

Clinical pharmacokinetics and pharmacodynamics of antimalarial combination therapy

Sofia Friberg Hietala

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UNIVERSITY OF GOTHENBURG

Department of Pharmacology

Institute of Neuroscience and Physiology

The Sahlgrenska Academy, University of Gothenburg,

Sweden

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To Albert

Abstract

In the face of growing drug resistance, the World Health Organization (WHO) has issued recommendations strongly encouraging the use of combination therapies to combat uncomplicated malaria. Amongst the most effective treatments are those combining an artemisinin derivative with a longer acting component such as amodiaquine, lumefantrine or piperaquine. Despite the widespread use of these treatments there is a lack of understanding regarding both pharmacokinetics and pharmacodynamics of the combinations, particularly in pediatric patients. The aim of this thesis was to describe how the dosing of antimalarials during combination therapy correlates with the outcome of treatment and to investigate factors that may influence this relationship.

In order to evaluate the pharmacokinetics and pharmacodynamics of the combinations artesunate + amodiaquine and artemether + lumefantrine in pediatric patients, a group particularly vulnerable to malaria, studies were conducted during the implementation of these new treatment strategies in Tanzania. The population approach to analysing the pharmacokinetics and pharmacodynamics was used in these studies. This method allows the determination of the typical values of pharmacokinetic and pharmacodynamic parameters, as well as the description of the variability in these estimates in the population, from sparse data. Importantly, the method also allows the investigation of how covariates, such as demographics (weight, age) or food intake influences pharmacokinetics and/or pharmacodynamics. An *in vitro* study was conducted to characterize the plasma protein binding of amodiaquine and its primary metabolite N-desethylamodiaquine. The influence of concomitant intake of a typical Vietnamese meal on the absorption of piperaquine was investigated in healthy subjects.

There was a significant, albeit weak, correlation between the clinical outcome of the combination amodiaquine+artesunate and exposure to N-desethylamodiaquine. Amodiaquine and N-desethylamodiaquine were both shown to be extensively bound to plasma proteins *in vitro*, which may explain the difficulty in establishing a good concentration-effect relationship from total N-desethylamodiaquine concentrations. The proposed semi-mechanistic model of parasite dynamics adequately described the effect of artemether and its active metabolite DHA on the parasite density in malaria patients, with predicted median parasite clearance time corresponding well with the observed. To make full use of the model, however, stage-specific parasite counts should be obtained both prior to, and during, drug treatment. There was no significant impact on the exposure to piperaquine due to concomitant intake of a relatively low-fat meal. The 20-fold range in exposure in both fed and fasting subjects suggests that there are other factors contributing significantly to interindividual differences in piperaquine pharmacokinetics.

Papers discussed

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

- I **Hietala SF**, Bhattarai A, Msellem M, Röshammar D, Ali AS, Strömberg J, Hombhanje FW, Kaneko A, Björkman A, Ashton M. Population pharmacokinetics of amodiaquine and desethylamodiaquine in pediatric patients with uncomplicated falciparum malaria. *J Pharmacokinet Pharmacodyn*. 2007 Oct;34(5):669-86. Reprinted with kind permission from Springer Science and Business Media
- II **Hietala SF**, Ahlin E and Ashton M. Binding of the antimalarial amodiaquine and its active metabolite N-desethylamodiaquine to albumin and α_1 -acid glycoprotein *in vitro* explain their binding in human plasma. *In manuscript*.
- III **Hietala SF**, Mårtensson A, Ngasala B, Dahlström S, Lindegårdh N, Annerberg A, Premji Z, Gil P, Björkman A, and Ashton M. Population pharmacokinetics and pharmacodynamics of artemether and lumefantrine in combination treatment of uncomplicated falciparum malaria in pediatric patients. *In manuscript*.
- IV Hai TN, **Hietala SF**, Van Huong N, Ashton M. The influence of food on the pharmacokinetics of piperazine in healthy Vietnamese volunteers. *Acta Tropica*. 2008 Aug;107(2):145-9. Reprinted with kind permission from Elsevier

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List of abbreviations

ACT	artemisinin based combination therapy
ARM	artemether
ARTS	artesunate
AQ	amodiaquine
AUC	area under the plasma concentration-time curve
AUC _{0-t}	AUC from time of dose until the time of the last quantifiable concentration
AUC _{t-∞}	AUC extrapolated from the last quantifiable concentration until infinity
AUC _{0-∞}	total AUC
AGP	α ₁ -acid glycoprotein
CL	clearance
CL/F	oral clearance
CL/F*fm	oral metabolite clearance
CL _{AQ}	amodiaquine clearance
CL _{ARM}	artemether clearance
CL _{DHA}	dihydroartemisinin clearance
CL _{DEAQ}	N-desethylamodiaquine clearance
CL _{LUM}	lumefantrine clearance
CI	confidence interval
C _{max}	maximum concentration
CV	coefficient of variation
CYP	cytochrome P450
DHA	dihydroartemisinin
F	bioavailability
FDA	American Food and Drug Administration
FOCE-I	first order conditional estimation with interaction
HPLC	high performance liquid chromatography
HSA	human serum albumin
IIV	interindividual variability
IOV	interoccasion variability
ka	rate of absorption
LLOQ	lower limit of quantification
LUM	lumefantrine
NCA	noncompartmental analysis
N-DEAQ	N-desethylamodiaquine
OFV	objective function value
PCT	parasite clearance time
PD	pharmacodynamics
PfATP6	parasite-encoded sarco-endoplasmic reticulum Ca ²⁺ -ATPase
PK	pharmacokinetics
PQ	piperaquine

Q	intercompartment clearance
$t_{1/2}$	half-life
V	Volume of distribution
V_c/F	Oral volume of distribution of the central compartment
V_p/F	Oral volume of distribution of the peripheral compartment
WHO	World Health Organization
λ_z	Slope of the terminal elimination phase

Introduction

Background

Half of the world's population is at risk of contracting malaria. Out of the estimated 200-300 million episodes in 2006, the vast majority (86%) occurred in Africa [1]. There were nearly a million malaria deaths the same year, of which 85% were children under the age of five [1]. In the face of growing drug resistance, the WHO has issued recommendations strongly encouraging the use of combination therapies to combat uncomplicated malaria [2]. Amongst the most effective treatments are those which combine an artemisinin derivative with a longer acting component [3, 4].

Optimal chemotherapy in malaria entail rapid, sustained clearance of parasites, short duration of treatment and low toxicity. The artemisinins, or endoperoxide antimalarials, have a fast parasitocidal action, a low toxicity and, it appears, a low potential for inducing resistance. In terms of optimal therapy, however, they fall short on one account. The efficacy of endoperoxide monotherapy is decreased by high recrudescence rates when administered for less than a week [5]. This is believed to be caused by the short half-lives, 1-4 hours, of the compounds [6]. To address the problem of recrudescence the endoperoxides are combined with other antimalarials with longer half-lives [7]. Here the endoperoxides are used to achieve a rapid decline in parasitemia, leaving only a small number of parasites to be killed by a longer acting drug.

Zanzibar, off the coast of East Africa, has opted for the combination of artesunate with amodiaquine while artemether and lumefantrine is first line treatment in mainland Tanzania. In Vietnam, South East Asia, dihydroartemisinin (DHA) and piperaquine is the therapy of choice for uncomplicated malaria. Despite the extensive use of artemisinin based combinations (ACTs) few dose-finding studies are available and dosing is based primarily on clinical experience. The aim of this thesis is to describe how the dosing of antimalarials during combination therapy correlates with the outcome of treatment and to investigate factors which may influence this relationship.

Malaria

Malaria is caused by the protozoan parasite *Plasmodium* and transmitted by mosquitoes. At least five species of the parasite have been shown to infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. While they share a basic life-cycle (Figure 1), certain distinctive features relate to the virulence of each species. *P. falciparum* causes the most severe manifestations of malaria including coma, anaemia and multi organ failure. The severity of *P. falciparum* infection has been attributed to the relatively high parasitemias during infection and to the adherence of *P. falciparum* infected erythrocytes to the endothelium of capillaries and venules, a process known as sequestration [8, 9]. Sequestered parasites are undetectable in peripheral blood and standard microscopy will underestimate the total parasite load in *falciparum* malaria. *P. vivax* and *P. ovale* form hypnozoites which linger dormant in the liver, and may cause clinical symptoms of malaria months to years after the initial infection.

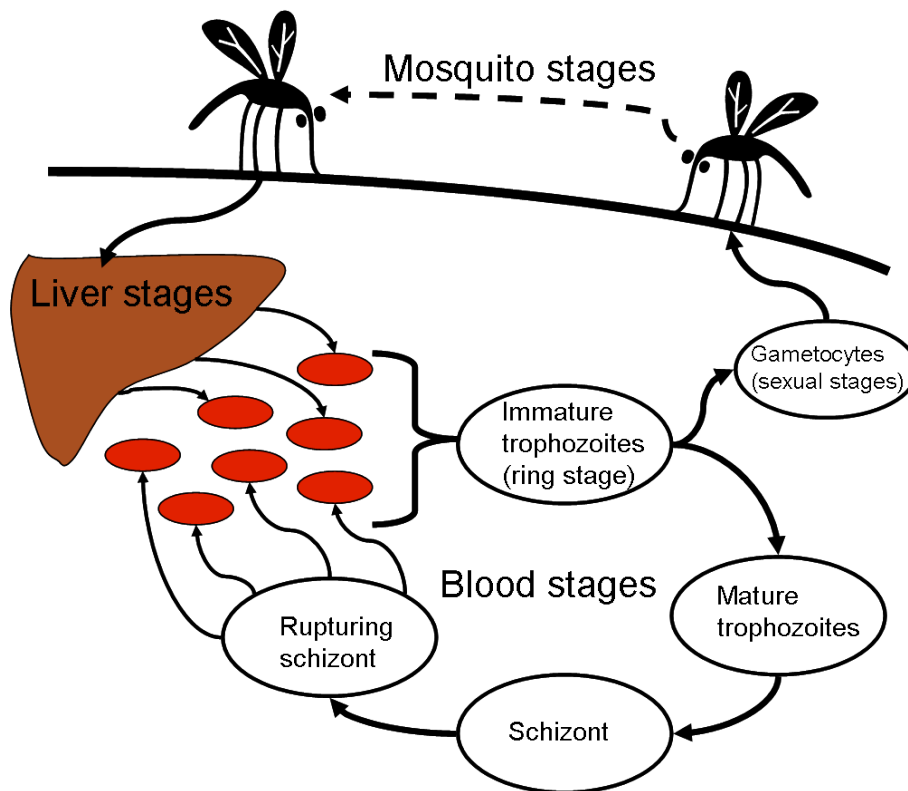


Figure 1. The malaria infection is initiated by the injection of *Plasmodium* sporozoites by the female *Anopheles* mosquito. Within minutes, the sporozoites infect liver cells where they mature into schizonts and rupture, releasing merozoites into the circulation. During the liver stage, which lasts one to two weeks, the infection is undetectable in peripheral blood. The merozoites infect red blood cells thus initiating the blood stage. Immature trophozoites, also known as early rings, develop into schizonts, rupture and reinfect new erythrocytes to complete the cycle of asexual reproduction. Some rings develop into the sexual form, gametocytes, which, if ingested by a mosquito, spread the disease. [10]

Artemisinin based combination therapy

Artemisinin antimalarials

Artemisinin, a sesquiterpene lactone, was first isolated from the herb *Artemisia annua* L., Sweet Wormwood, in 1972 [11]. The artemisinins are also termed endoperoxides for the presumptive pharmacophore: an endoperoxide bridge. The endoperoxide moiety is believed to interact with intraparasitic heme to form reactive C-centered radicals that disrupt parasite proteins [12]. A major target appears to be the parasite-encoded sarco-endoplasmic reticulum Ca^{2+} -ATPase (PfATP6) [13].

Artesunate and artemether are semi synthetic derivatives of artemisinin. While artesunate is water soluble and suitable for all routes of administration (oral, rectal, intramuscular and intravenous) artemether is lipophilic and unsuitable for intravenous use. Both artesunate and artemether are readily absorbed from the gastrointestinal tract. The oral bioavailability of artesunate is low due to the rapid and extensive conversion to DHA [14, 15]. DHA is further metabolized through glucuronidation [16] with a reported elimination half-life ranging from 0.4 to 12.5 hours [17][18, 19]. Artemether is eliminated through Cytochrome P450 (CYP)-mediated demethylation to DHA with a half-life of 1 to 4 hours [18-23]. Artemether, like artemisinin, has capacity for autoinduction [22]. While intestinal CYP3A4 appears involved in the first-pass metabolism of artemether, it does not account for the autoinduction of metabolism [24, 25].

Current daily oral doses of artesunate, artemether and DHA, in combination with longer acting drugs, range from 2.5 to 4 mg/kg [4]. The effect of varying the amount of artesunate, administered as a single dose together with mefloquine, on parasite clearance time (PCT) showed no increased shortening of PCT with doses in excess of 2 mg/kg [26].

Amodiaquine

Amodiaquine is a 4-aminoquinoline similar to chloroquine. The 4-aminoquinolines act by inhibiting the degradation of haemoglobin in the food vacuole of the parasite [27].

Amodiaquine is rapidly metabolized to the active metabolite N-desethylamodiaquine (N-DEAQ) through a reaction catalyzed by CYP2C8 [28].

While the half-life of amodiaquine is approximately 4 hours in adults, N-DEAQ has a considerably longer terminal half-life ranging from 3 to 18 days in adults [29-31]. The main route of elimination of N-DEAQ is unknown. Further metabolism to bis-DEAQ has been suggested although the plasma and urine concentrations of this metabolite are low [32].

Both amodiaquine and N-DEAQ possess antimalarial activity *in vitro* [33, 34]. Due to the rapid conversion of amodiaquine to N-DEAQ the metabolite is assumed to be responsible for the main clinical effect. *In vitro* studies, however, suggest a synergism between amodiaquine and N-DEAQ [35].

Amodiaquine has been implicated as the cause of serious adverse reactions, agranulocytosis and hepatotoxicity, during prophylactic use [36]. A difference in the frequency and severity of adverse events during amodiaquine treatment of malaria compared to other common therapies (chloroquine, sulfadoxine-pyrimethamine) has, however, not been demonstrated [37].

Few studies have addressed the optimal dosing of amodiaquine in combination with artesunate in malaria treatment. A retrospective analysis of the effect and safety of various amodiaquine doses resulted in a suggested therapeutic dose window of 7.5 to 15 mg/kg for three consecutive days [38].

Lumefantrine

Lumefantrine is a fluorene derivative discovered at the Academy of Military Medical Sciences in China. The mechanism of action of lumefantrine is unclear. Lumefantrine is slowly absorbed with an estimated absorption half-life of 5 hours. The maximum concentration is reached in approximately 10 hours [20, 39]. The bioavailability of lumefantrine has been shown to be influenced by concomitant administration of food, or a fatty drink [20, 40]. The elimination of lumefantrine is slow and the terminal half-life is 3-6 days in adults [20]. Lumefantrine has been shown to be metabolized by CYP3A4 and may interact with other drugs metabolized by the same enzyme [41].

A correlation between the exposure to lumefantrine and the chance of radical cure has been shown [20]. The 28-day cure rate of the combination has been associated with the body weight normalized dose [42]. Increasing exposure to lumefantrine has been associated with a greater chance of radical cure but did not explain variability in parasite clearance time (PCT). In contrast, increasing exposure to artemether and DHA both caused a decrease in PCT, but did not significantly influence the cure rate. [20]

Piperaquine

Piperaquine was first synthesized at Rhone Poulenc, France, in the 1950s as compound 13228 RP [43], but was abandoned due to lack of commercial interest. It was later produced by the Shanghai Research Institute of Pharmaceutical Industry in 1966 under the name piperaquine.

Piperaquine disposition is characterized by a multiphasic profile with an exceptionally long terminal half-life which may exceed one month in the adult [44-49]. The piperaquine concentrations sustained on a 20-50 µg/L level may contribute to a post-treatment prophylactic effect [50] lasting several weeks. Plasma profiles of piperaquine exhibit multiple peaks [51-53]. Studies in healthy volunteers have suggested an effect of food on the exposure to piperaquine [51, 54].

Pharmacokinetics and pharmacodynamics

Pharmacokinetics

Pharmacokinetics (PK) is the study of the fate of pharmaceutical compounds in the body. Some basic PK parameters govern the time course of exposure to orally administered drugs, namely: bioavailability (F), absorption rate constant (k_a), clearance (CL) and volume of distribution (V). There are several ways to study and describe PK. The choice of method depends primarily on the aim of the investigation but may be influenced by the possibility to obtain adequate data and/or prior knowledge regarding the substance at hand.

In noncompartmental analysis (NCA), the total exposure, expressed as area under the concentration-time curve (AUC), is used to estimate the PK parameters. The method makes no assumption regarding the shape of the exposure time curve, but the accuracy is highly dependent on an adequate sampling frequency.

Another approach to PK analysis is the compartmental analysis. This model based method identifies the shape of the exposure (one-, two- or multicompartmental) and estimates the parameters accordingly. An advantage of this approach is that it requires less frequent sampling. A further benefit of a model based analysis is that it allows for the prediction of drug concentration profiles that would arise from various dosing schedules.

In the population approach to PK analysis, the statistical method termed mixed-effects modelling is used to describe not only the central tendency in parameters, but also the spread of parameter values in the population [55, 56]. Ideally, the analysis also allows the description of the underlying cause of the variability in parameters, i.e. the identification of covariates. In a mixed-effects model, observations are thought to be the sum of the so-called fixed and random effects. Fixed effects are those influencing the typical estimate of a parameter in a population (similar to a mean or a median), while random effects represent unexplained, seemingly random, variability in observations and parameter estimates.

For a drug exhibiting one-compartment PK the observed concentration at a given time following intravenous administration, C_{ti} , could be described by:

$$C_{ti} = \frac{Dose}{V_i} \exp^{-\frac{CL_i}{V_i} \times t} + \varepsilon_{ti}$$

where CL_i and V_i is the individual clearance and volume of distribution respectively, ε represents a random error term (with mean 0 and variance σ^2) describing the difference between the predicted concentration and the observed. ε may result from model misspecification, erroneous concentration determinations or sampling error.

The parameter estimate for the individual subject is described by the typical value of the parameter in the population, and a variability term, η_i (with mean 0 and variance σ^2), allowing for differences between patients.

$$CL_i = TVCL \times \exp^{\eta_i}$$

Many pharmacokinetic parameters are log-normally distributed in the population and the variability is best described by an exponential variance term as shown above.

Effect models

The intensity and the time course of drug effects, pharmacodynamics (PD), can be described through the application of mathematical models. PD models can be more or less mechanistic in nature. The logistic regression model in paper I is an example of an empirical, non-mechanistic model. While describing the correlation between exposure to N-DEAQ and the risk of parasitemia during follow up it does not allow the further investigation of the nature of the effect of N-DEAQ on the disease parameters. The model in paper III is a semi-mechanistic model based on the life-cycle of *P. falciparum*.

Some early models of the within-host dynamics of *P. falciparum* describe the development of parasitemia in terms of infected and uninfected red blood cells and merozoites. In the absence of host immunity, these models predict the growth of parasitemia to depend on the replication rate at merogony and access to uninfected red blood cells [57, 58]. More recent work has shown that the number of red cells is unlikely to limit the propagation of parasitemia [59, 60]. A number of PD models based on the erythrocytic life-cycle of *P. falciparum* have been presented [61-64]. Common features of these models are the description of the age-stages of *P. falciparum* and the division of the parasite population into circulating and sequestered parasites. This approach allows for the description of potentially stage-specific drug action as well as renders estimates of the total, rather than visible, parasite load.

Investigating variability in PK and PD

In order to optimize dosing it is important to identify sources of significant variability in PK and PD parameters. In the two stage PK analysis, PK parameters are estimated for each individual separately (through compartmental or non-compartmental approaches) and then grouped to allow the investigation of the correlation between PK and subjects specific factors. In population analysis, covariates can be incorporated into the expression for a parameter during the model building process.

Covariates can be demographic factors, patient's age and weight, biochemistry ($S_{\text{creatinine}}$ and plasma proteins) or behavioural factors such as the timing of food intake in relation to drug administration. While patient size rarely contributes significantly to interpatient variability within the adult population, pediatric dosing often requires adjustment according to size. Traditionally, body weight or body surface area has been used to scale the dose in children, however, allometric scaling based on the correlation between the log of the basal metabolic rate and the log of the body weight is gaining ground [65, 66]. A further challenge in pediatric dose optimization is to adequately account for age related changes in renal and metabolic function. For relatively narrow age spans linear models often suffice to describe the development of the eliminating organs, while more complex exponential, or gradual models, are required for wider age ranges [65].

Drug binding to plasma proteins

Even though it is generally accepted that the unbound concentration of drug in blood or plasma best describes the effective concentration, most clinical studies assess PK from total concentrations. As the protein binding of a drug affects PK parameters derived from total drug concentrations, inter- and intra-individual variability in protein concentration may significantly influence PK for substances highly bound in plasma.

The primary drug binding proteins in plasma are albumin, α_1 -acid glycoprotein (AGP), and lipoproteins. While the albumin concentration is maintained at a relatively constant level of 500-700 μM , AGP is an acute phase reactant elevated in response to inflammatory processes. AGP concentrations have been shown to increase from the normal 10-20 μM to 60 μM during acute malaria infection [67, 68]. The concentrations of lipoproteins varies in disease states, but are also influenced by dietary fat intake. A high fat meal causes transient hyperlipidemia [69]. A study comparing the plasma lipids in 60 malaria patients to those in healthy subjects indicated significantly elevated concentrations of lipoproteins during malaria infection, except regarding triglycerides, which were significantly lower in severe malaria patients [70].

The protein binding has been shown to influence the PK of a number of antimalarials. The distribution of primaquine to red blood cells is inversely proportional to the concentration of AGP [71]. The CL and V of quinine increase during recovery from malaria infection in children and adults, a change that has been attributed to the decrease in AGP concentration during this time [67, 72]. The exposure to halofantrine, a highly lipid-bound antimalarial, increases with concomitant food intake. *In vivo* studies in dogs and rats have indicated that this change in PK result from an increased binding capacity of lipoproteins in the postprandial state [73, 74].

Food-drug interactions

Concomitant food intake may alter both PK and PD. Commonly recognized mechanisms of food-drug interactions include the chelate formation that lead to reduced bioavailability of tetracyclines with milk [75] and the hypertensive crisis caused by the combination of monoaminoxidase-inhibitors with tyramin rich foods such as aged cheese and red wine [76]. In recent years the influence of grapefruit juice on intestinal metabolism and absorption has rendered attention. Grapefruit juice has been shown to increase the bioavailability of, amongst other drugs, artemether and felodipine through inhibition of CYP3A4 in the gut lumen [24, 77]. However, fruit juices have also been shown to counteract absorption through inhibition of organic anion transporting polypeptides [78]. Changes in presystemic metabolism or absorption result in altered concentrations of free drug in the systemic circulation, and thus cause clinically relevant interactions.

The PK of the lipophilic antimalarial lumefantrine has been shown to be affected by concomitant food intake. A study in healthy subjects showed a dramatic, 16-fold, increase in the lumefantrine AUC following a high fat meal [79]. This food-drug interaction was recently confirmed by Ashley and colleagues [40], who showed the increase in AUC to be dependent on the amount of fat taken with the lumefantrine dose. Lumefantrine, like halofantrine, binds extensively to lipoproteins [80], and the mechanism for the increased exposure with food is unclear.

Other lipophilic antimalarials potentially affected by concomitant food intake include piperaquine and mefloquine, however the extent of these effects varies between studies [51, 81, 82]. Combining piperaquine with a high fat meal resulted in a two-fold increase of the total exposure as well as a marked increase in maximum concentrations (C_{max}) in healthy Caucasian volunteers while a meal with a lower fat-content showed a smaller increase in the piperaquine concentration (40%) in Vietnamese volunteers [54]. The clinical implication of an increased exposure due to food intake is unclear. It has been proposed that an increased exposure due to better absorption with food may cause a greater risk for side-effects rather than increase efficacy [51].

Specific aims

The aim of this thesis was to describe how the dosing of antimalarials during combination therapy correlates with the outcome of treatment and to investigate factors that may influence this relationship, specifically:

- The pharmacokinetics of amodiaquine and its primary active metabolite N-DEAQ in pediatric patients and the correlation between pharmacokinetics of N-DEAQ and the clinical outcome of combination treatment of amodiaquine and artesunate.
- The extent and nature of plasma protein binding of amodiaquine and N-DEAQ.
- The pharmacokinetics and pharmacodynamics of artemether, DHA and lumefantrine in combination during treatment of uncomplicated falciparum malaria in pediatric patients.
- The effect of food on the pharmacokinetics of piperaquine in healthy volunteers.

Methods

Ethics

Data from six clinical studies were included in this thesis. All studies were conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The studies in paper I were approved by The Ethics committees at Karolinska Institute and Göteborg University, Sweden, The Zanzibar Ministry of Health, Zanzibar, Tanzania, National Department of Health Medical Research Advisory Committee, Papua New Guinea and Tokyo Women's Medical University Ethical Committee, Japan. The studies in paper III were approved by the Ethics committee at Karolinska Institute, Sweden and The Ministry of Health, Tanzania. The study in paper IV was approved by The Ministry of Health, Vietnam.

Patient/subject inclusion and sampling schedules

Population pharmacokinetics and pharmacodynamics of amodiaquine and desethylamodiaquine in pediatric patients

Drug concentrations from a total of 244 patients (aged 3 months to 12 years) in three clinical studies, two conducted in Zanzibar and one in Papua New Guinea, were included in the analysis. All patients had microscopically confirmed falciparum malaria.

In the Zanzibar studies doses of amodiaquine and artesunate, (Arsucam®, Creapharm, France) were determined on the basis of age, in accordance with the national treatment policy. Patients younger than 1 year received 25 mg of artesunate and 50 mg of amodiaquine-HCl (equivalent to 38.3 mg amodiaquine), patients aged 1-6 years received 50 mg of artesunate and 100 mg of amodiaquine-HCl (equivalent to 76.5 mg amodiaquine) and patients aged 7-12 years received 100 mg of artesunate and 300 mg of amodiaquine-HCl (equal to 229.5 mg amodiaquine) once daily for 3 consecutive days. Capillary blood samples for drug concentration analyses were obtained from all subjects on days 7 and 14 (n=224). More frequent samples were obtained from 12 patients according to one of following schedules: 0 (pre-dose), 0.25, 1, 3, 5 and 7 hours or 0 (pre-dose), 0.5, 2, 4, 6 and 8 hours after start of treatment.

The patients in Papua New Guinea received 10 mg/kg/day of amodiaquine (infant Camoquin®, Prawll Laboratories Ltd, India, 100 mg tablet) for 3 days and a single

dose of sulphadoxine-pyrimethamine on day 7 (25 mg/kg, based on the sulphadoxine component). Amodiaquine and N-DEAQ concentrations were determined at 0 (pre-dose), 2, 4, 12, 24, 36 and 48 hours following treatment initiation and on days 3, 5, 7 and 14 [83].

Population pharmacokinetics and pharmacodynamics of artemether in combination with lumefantrine in pediatric patients

A total of 50 patients, aged 1-10 years, suffering from microscopically confirmed falciparum malaria with fever were included in the study. Patients were excluded if they had haemoglobin below 70 g/L, were suffering from severe malnutrition or showed signs of severe malaria. Weight-based doses of Coartem[®] containing 20 mg artemether and 120 mg lumefantrine (Novartis Pharma Ltd, Switzerland) were administered at 0, 8, 24, 36, 48 and 60 hours. Patients weighing 5-14 kg received one tablet/dose, patients weighing 15-24 received two tablets/dose and patients weighing 25-34 kg received three tablets/dose. Patients were randomized to ingest the drug dose with a glass of milk (200 ml) (n=25), or to take the medicine with water (n=25).

Venous blood samples for drug concentration analyses and for determination of parasitemia were obtained pre-dose (-2 hours) and at 0, 2, 4, 8, 16, 24, 36, 48, 60 and 72 hours following treatment initiation.

Data from two previously published studies of the parasite densities in peripheral blood from asymptomatic children in a similar coastal setting in Tanzania were included in the parasite growth model [84, 85].

The influence of food on the pharmacokinetics of piperazine

Thirty-two healthy Vietnamese adult subjects were included in the study. Following an overnight fast subjects received two tablets of CV.Artecan (Pharmaceutical Company 26, Ho Chi Minh City, Vietnam), each containing 40 mg DHA and 320 mg piperazine phosphate (equivalent of 171.5 mg of piperazine base) as a single dose. Subjects were randomly assigned to take the study drugs together with a standardized Vietnamese meal (n=16) or to remain fasting for another four hours following drug intake (n=16). The meal consisted of one fried egg and a meat soup (pork (0.1 kg), beef (0.1 kg), rice, vegetables and beans) and contained approximately 17 g fat, 16 g protein and 53 g carbohydrates. Blood samples were obtained through an indwelling venous catheter at -5 min (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 28, 32 and 36 hrs after dose and by venepuncture in the mornings on days 7, 14, 21, 28, 35, 42, 49.

Biochemical analyses

The methods of drug concentration analyses are specified in each paper I-IV.

The parasitemias in papers I and III were determined from Giemsa stained thick blood films. Asexual parasite density was calculated against 200 (paper I) or 500 (paper III) white blood cells, assuming a white blood cell count of 8000/ μ l. If less than 10 parasites were detected per 200 white blood cells, estimates were made against another 300 white blood cells. Slides were prepared and examined at the study sites.

Albumin and AGP were quantified at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Göteborg, Sweden (paper II).

Population pharmacokinetic and pharmacodynamic modelling

The population PK and PD were modelled using NONMEM version V and VI (Icon Development Solutions, Maryland, USA) under Compaq Visual Fortran version 6.6. The first order conditional estimation method (FOCE) or the FOCE with interaction (FOCE-I) was used in PK and PD model building, except for the logistic model in paper I where the FOCE with the Laplacian option was used. Homoscedastic and heteroscedastic error models were tested to describe residual error. Variability was estimated as interindividual variability (IIV) and as interoccasion variability (IOV).

Covariate effects were identified using the general additive method (GAM) as implemented in Xpose Version 4.0 [86]. The likelihood ratio test (LRT), i.e. the difference in the value of the objective function (OFV) was used to statistically evaluate the performance of nested models [55, 87]. However, failure of a covariate to explain interindividual variability resulted in exclusion from the final model. The predictive performance of the models were assessed with visual predictive checks as described by Holford [88].

Equilibrium dialysis to determine plasma protein binding

Albumin solutions were prepared by diluting human serum albumin (HSA) (Baxter, Chicago, USA) with isotonic phosphate buffer pH 7.4 to give HSA concentrations of 300, 400, 500, 600 and 1000 μ M. An AGP solution was prepared from freeze dried human AGP (Sigma Aldrich, Stockholm, Sweden) at a concentration of 23 μ M. Plasma from healthy donors was obtained from Sahlgrenska University Hospital, Gothenburg, Sweden. Amodiaquine and N-DEAQ, dissolved in isotonic phosphate buffer pH 7.4 was added to concentrations 250 nM, 500 nM, 1000 nM, 2000 nM and 4000 nM in the protein matrices.

Equilibrium dialysis cells (Scienceware, Belart Products, Pequannock, USA) with a semi permeable cellulose membrane (molecular weight cut-off \sim 6000 Da, Scienceware, Belart Products, Pequannock, USA), were used in the experiments.

Equal volumes (1.00 mL) of drug-spiked protein containing medium (human plasma, HSA and AGP-solutions) and isotonic phosphate buffer (pH 7.4) were introduced on either side of the membrane. Cells were incubated in a slowly agitating water bath at 37°C for 7 hours for plasma and HSA and 3 hours for AGP. Following incubation, samples of both the total concentrations (protein matrix) and unbound concentrations (in isotonic buffer) were analyzed.

The binding properties, expressed as binding affinity, K_{aff} , of amodiaquine and N-DEAQ to HSA and AGP were modelled in WinNonlin version 5 (Pharsight, Mountain View, California, USA), according to:

$$[D]_{Proteinmatrix} = [D]_{Buffer} + [P] \times k_{aff} \times [D]_{Buffer}$$

where $[D]_{Proteinmatrix}$ and $[D]_{Buffer}$ are the total and unbound drug concentrations of amodiaquine and N-DEAQ, respectively, and $[P]$ the total protein concentration in the protein solutions. As alternative models, one incorporating drug concentration dependent binding and another adding a linear term for unspecific binding, were tested and discarded.

Noncompartmental analysis

The pharmacokinetic parameters of piperazine were determined by NCA as recommended by the FDA [89] in WinNonlin version 5 (Pharsight, Mountain View, California, USA). The AUC was calculated using linear interpolation between increasing concentrations and logarithmic interpolation between declining concentrations.

The AUC_{0-last} was defined as the area under the concentration time curve from the time of dose until the last concentration above LLOQ. The terminal half-life, $t_{1/2\lambda}$ was calculated as $\ln 2 / \lambda_z$, where λ_z is the slope of the terminal phase. The $AUC_{last-\infty}$ was extrapolated from the predicted concentration (C_{pred}) at the time of the last concentration above the LLOQ ($AUC_{t-\infty} = C_{pred} / \lambda_z$).

The median and the 80% central range of the pharmacokinetic parameters were calculated in Microsoft Excel (Microsoft Corporation, Washington, USA). The estimated pharmacokinetic parameters for the fed and the fasting state were compared using the Mann-Whitney two sample rank-sum test in SPSS 12.0.1 for Windows (SPSS Inc. Illinois, USA).

Results

Population pharmacokinetics of amodiaquine and N-desethylamodiaquine (paper I)

The pharmacokinetics of amodiaquine and N-DEAQ were best described by two parallel two-compartment models with a central and a peripheral compartment for each compound and a shared estimate of k_a . A model describing the absorption of amodiaquine and the formation of N-DEAQ as consecutive processes did not converge. Simultaneous introduction of amodiaquine and N-DEAQ from separate dosing compartments better described the data. The typical parameter estimates for amodiaquine and the relative standard errors of the estimates (RSE) were oral clearance (CL/F) 14 (8%) L/h/kg, oral volume of distribution of the central compartment (V_c/F) 11.7 (91%) L/kg, intercompartment clearance (Q) 17 (28%) L/kg and oral volume of distribution of the central compartment (V_p/F) 311 (18%) L/kg. The residual error was best described by a proportional error of 41% and a fixed additive error of 25 nM. The fit of the PK model is illustrated in Figure 2.

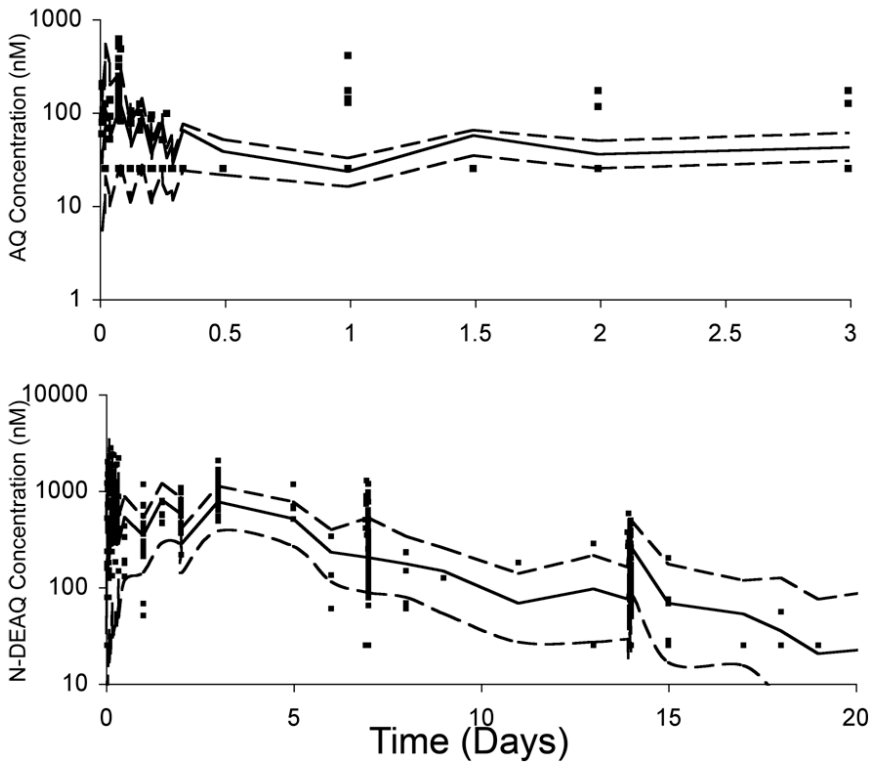


Figure 2. The visual predictive check illustrating the distribution of observed amodiaquine (first panel) and N-DEAQ (second panel) concentrations in relation to their respective simulated 90% prediction interval.

The inclusion of age as a predictor on body weight normalized oral metabolite CL_{N-DEAQ} ($CL/F \cdot F_m$) significantly reduced the OFV ($\Delta_{OFV} = -12.3$). The relationship was modelled with the function:

$$\frac{TVCL_{N-DEAQ}}{F \cdot fm} = 0.67 \exp^{-0.006 \times AGE}$$

Remaining typical parameter estimates for N-DEAQ were, V_c/F 12.8 (44%) L/kg, Q 1.3 (23%) L/kg and V_p/F 62.4 (9%) L/kg. The residual error was described by a proportional error of 49% and a fixed additive error of 25 nM. There was some variability in the terminal elimination half-life of N-DEAQ in the study populations. The mean terminal elimination half-life was 125 ± 32 (mean \pm SD) and 183 ± 57 h for the patients in Zanzibar and Papua New Guinea, respectively.

Correlation between drug exposure and parasitemia during follow-up (paper I)

An association between N-DEAQ concentration on day 7 and the risk of parasitemia within one month of treatment was identified. The probability of having parasitemia during the first month following treatment was best described by the function:

$$P(\text{Parasitemia}_i) = \frac{e^{-0.4 - 0.004 \times C_{N-DEAQ,d7_i}}}{1 + e^{-0.4 - 0.004 \times C_{N-DEAQ,d7_i}}}$$

The model estimated risk of having parasitemia during follow up was 40% in patients with undetectable N-DEAQ concentration on day 7. As illustrated in Figure 3, this corresponds well with the observed probability of 45%. The inclusion of age, initial parasitemia, observed N-DEAQ concentration on day 14, AUC, C_{max} or $t_{1/2}$, did not improve the logistic model.

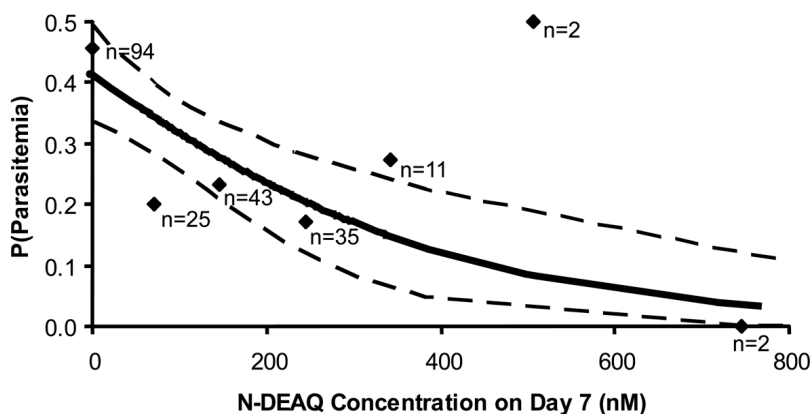


Figure 3. Model predicted (solid line) and observed risk of recurring parasitemia against N-DEAQ concentration on day 7. Observations are binned in increments of 100 nM. The broken lines represent the 90% prediction interval of the probability curve from 1,000 bootstrapped datasets.

The protein binding of amodiaquine and N-desethylamodiaquine (paper II)

Amodiaquine and N-DEAQ were shown to be extensively bound to plasma proteins with mean (SD) observed bound fraction of $92\pm 3\%$ and $85\pm 7\%$ respectively in plasma from healthy subjects. Binding to both albumin and AGP were non-saturable at clinical concentrations of amodiaquine and N-DEAQ. The total concentration of amodiaquine and N-DEAQ in plasma was described by the following functions:

$$[AQ]_{ot} = [AQ]_f + [426]_{HSA} \times [AQ]_f \times 0.015 + [9.4]_{AGP} \times [AQ]_f \times 0.13$$

$$[DEAQ]_{ot} = [DEAQ]_f + [426]_{HSA} \times [DEAQ]_f \times 0.008 + [9.4]_{AGP} \times [DEAQ]_f \times 0.30$$

The change in f_u for amodiaquine and N-DEAQ depending on altered concentration of AGP was simulated using the following function, and illustrated in Figure 4:

$$f_u = \frac{1}{1 + [P]_{HSA} \times K_{aff,HSA} + [P]_{AGP} \times K_{aff,AGP}}$$

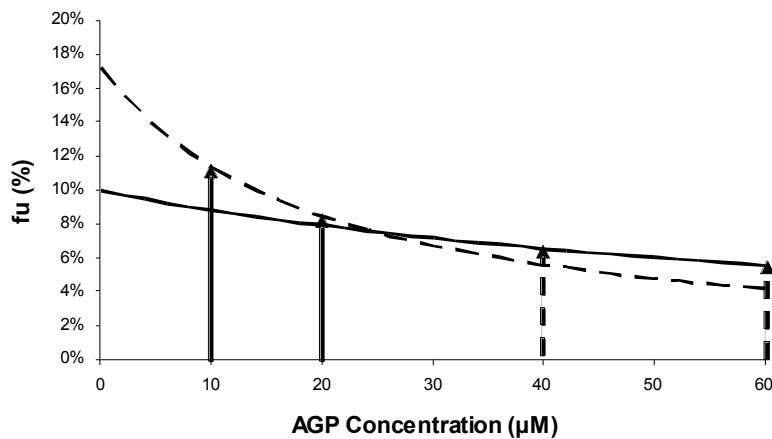


Figure 4. Simulated unbound fractions of amodiaquine (solid line) and N-DEAQ (dashed line) versus AGP concentration. HSA concentrations were kept constant at $600 \mu\text{M}$. While the normal concentration of AGP is $10\text{-}20 \mu\text{M}$ (black arrows), this may rise to $40\text{-}60 \mu\text{M}$ during acute malaria infection (dashed arrows)

The pharmacokinetics and pharmacodynamics of artemether, dihydroartemisinin and lumefantrine (paper III)

A two-compartment model with first order absorption best described the distribution of artemether. The bioavailability of both artemether and DHA was fixed to 1 to make the model identifiable. Due to limited sampling during absorption k_a was fixed to 1/h in the final model. Altering the rate of absorption between 0.2 and 2/h did not significantly influence the remaining parameter estimates. There was a time-dependency in artemether kinetics described by occasion as a covariate on CL_{ARM} . The CL_{ARM} was estimated to 2.6 L/h/kg for the first dose and increases by a fraction of 0.57 with each dose occasion with a CV of 41%. A covariate-free one-compartment distribution model adequately illustrated the DHA concentrations. The CL_{DHA} was 6.8 L/h/kg with a CV of 47%.

The PK of lumefantrine was best described by a one-compartment model with an absorption lag time. The CL_{LUM} was estimated to 77 mL/h/kg with a CV of 82%. The inclusion of concomitant milk intake as a covariate on PK parameters did not significantly improve the model.

The performance of the PK models is illustrated in figure 5.

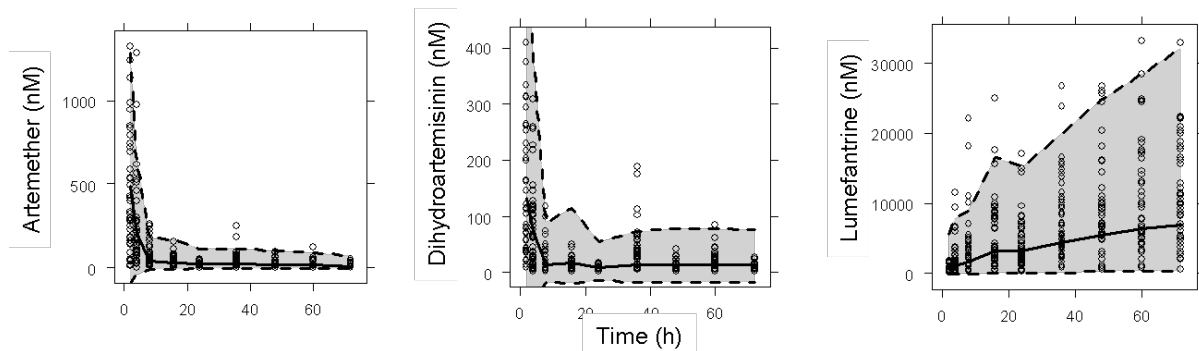


Figure 5. Visual predictive checks of the pharmacokinetic models. The open circles are the observed concentrations. The shaded area represent the 95% prediction interval, calculated from simulated observations from 1000 studies, and the solid line represents the median of the predictions.

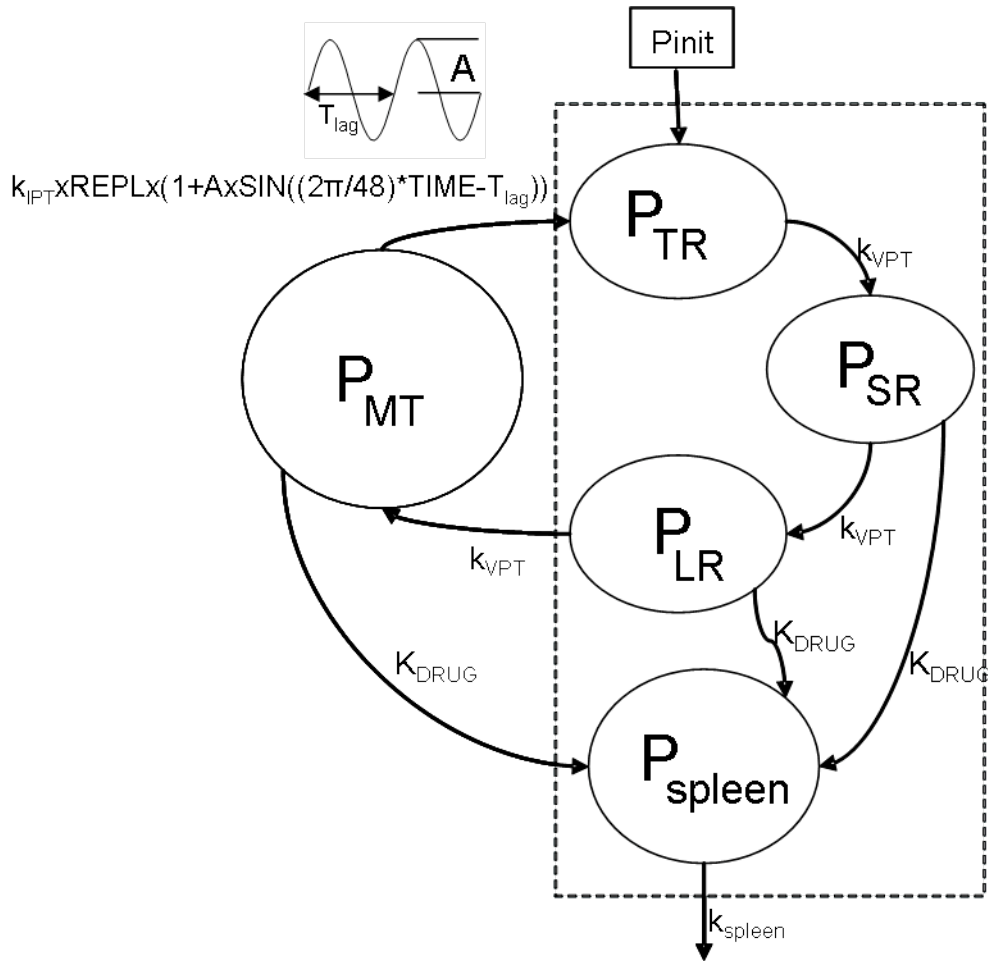


Figure 6. The pharmacodynamic model based on the blood stages of *P. falciparum*. Compartments within the dashed rectangle represent parasites visible in peripheral blood.

The PD model (Figure 6) is initiated by the introduction of a fixed number of parasites, P_{init} , into the compartment denoted P_{TR} representing parasites in the earliest ring stage. The parasites mature through the erythrocytic stages: tiny rings, small rings, large rings and mature trophozoits/schizonts. The covariance step in NONMEM was not completed for the final PD model and the uncertainty in parameter estimates is reported as the 95% confidence interval for the estimates from 100 non-parametric bootstrap samples.

The mean transit time, MTT, through the asexual cycle was estimated to 48.5 (48.2; 48.7) hours, and the mean age at sequestration, VPT, was estimated to 17 (9.9; 24.6) hours. The rate constants governing the development of the parasites through the cycle are described by $k_{VPT} = 3/VPT$ and $k_{IPT} = 1/(MTT - VPT)$. Parasites that are killed or injured due to drug action (k_{DRUG}) are assumed to remain in the blood until removed by the spleen or macrophages. These parasites are represented by the compartment denoted P_{spleen} . Multiplication occurs from schizonts to tiny rings, with a factor denoted REPL and estimated to 0.8 (0.44; 0.96) and 4.6 (2.9; 12) in asymptomatic and symptomatic children respectively. To account for synchronicity

in the parasite population a sine function is applied to the P_{TR} compartment. The amplitude, A , of the sine function determines the fluctuations in parasitemia over time. The sine function was not supported in the data from symptomatic patients. The pre-treatment dynamics of the parasite population were estimated from the data obtained in asymptomatic individuals and in patients prior to dosing. Parameter estimates for P_{init} , Lag , MTT , VPT , A were then fixed prior to modelling of drug effects.

The drug effects were modelled sequentially beginning with artemether and DHA that have been shown to contribute most significantly to the immediate decline in parasitemia [90]. Artemether and DHA affect multiple stages of parasite development and appear to have a similar potency [91-93]. The effects of artemether and DHA were modelled on all developmental stages as:

$$k_{ARM} = S_{ARM/DHA} \times \log[ARM]$$

$$k_{DHA} = S_{ARM/DHA} \times \log[DHA]$$

where $S_{ARM/DHA}$ is the slope of both the artemether and DHA concentration-effect curves estimated to 0.029 (0.026; 0.030). There was a time delay in the artemether/DHA concentration-effect correlation modelled with a lag-time.

The introduction of an effect of lumefantrine did not significantly improve the model fit, and the parameter estimates for the lumefantrine effect could not be estimated. The fit of the PD model is illustrated in Figure 7.

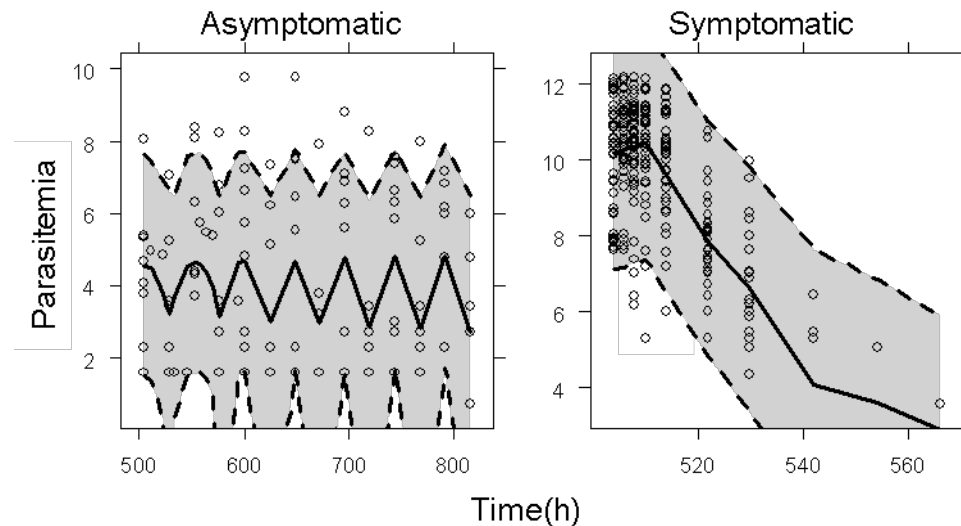


Figure 7. Visual predictive check of the pharmacodynamic model. The open circles are the observed parasitemias. The shaded area represents the 95% prediction interval, calculated from simulated observations from 1000 studies and the solid lines represent the simulated median parasitemia.

The effect of food on the pharmacokinetics of piperazine (paper IV)

The median (80% central range) AUC_{0-last} was 11.5 (6.9-17.3) h×mg/L in fed and 13.9 (2.8-19.3) h×mg/L in fasting subjects, indicating a considerable variability in exposure in both groups. There was no statistically significant difference in exposure between fed and fasting subjects.

The estimated overall oral CL was 0.27 (0.12-1.49) L/h/kg, the V during the terminal elimination phase was 230 (102-419) L/kg and estimated $t_{1/2\lambda}$ was 18 (5-93) days. The multiple peaks described also in previous studies occurred in both groups [45, 48, 49].

Discussion

Treatment of uncomplicated malaria, particularly in children, is a life-saving intervention. Prompt and adequate management of the disease is important to avoid the development of severe illness and to minimize spreading of the disease [94]. While numerous studies compare different treatments, very few have addressed the dose-effect correlation of the respective drugs. The aim of this thesis was to describe how the dosing of antimalarials during combination therapy correlates with the outcome of treatment and to investigate factors that may influence this relationship.

The use of PK-PD modelling to elucidate the correlation between the dosing of a drug and the clinical effect is gaining ground [95, 96]. The population approach is one such pharmacometric tool, particularly useful in the analysis of sparse data. In papers I and III the population approach was used to evaluate the PK and PK-PD of combination treatments in children, the patient population most vulnerable to malaria [1]. Drug therapy in pediatric patients is often based on clinical experiences in adults, and dosing in children is designed to mimic the drug exposure in adults [66, 97]. The PK determinants of exposure are F and CL . Studies have shown that the size normalized CL often differs between children and adults rendering body size alone inapt for dose normalization [66, 98, 99]. A negative correlation between age and weight normalized CL in pediatric patients has been described for several drugs and is likely to be explained by the nonlinearity in the relationship between the function of the eliminating organs (liver and kidneys) and body weight [100]. In consequence doses normalized to body weight should rather be greater in children than in adults. The correlation between the drug concentration and both clinical and unwanted effects may also be age dependent [101]. The complex development of partial immunity to malaria is likely to contribute to age related changes in treatment outcome [97].

There was a statistically significant, although weak, correlation between N-DEAQ exposure, described by concentration on day 7, and the risk of recurrent parasitemia within a month of treatment initiation. The body weight normalized CL_{N-DEAQ} in pediatric patients was shown to be nonlinearly related to body weight, resulting in a higher bodyweight normalized CL in smaller children. In the studies conducted in Zanzibar, the dosing of amodiaquine was based on age in accordance with the national treatment policy and as recommended in the WHO guideline [2]. Resulting mean dose per body weight was 7.4 mg/kg/day in the small PK study where actual body weights were recorded and 6.2 mg/kg/day based on weight for age calculated body weights for remaining patients. In both groups the average dose was lower

than the described target dose of 7.5–15 mg/kg/day [25]. Further, only five patients in the PK study, with relatively frequent sampling, had concentrations above 135 ng/ml on day 4, the cut-off concentration associated with a positive outcome of amodiaquine monotherapy according to Aubouy and colleagues [11]. These findings indicate that the currently recommended age based dosing in Zanzibar may result in inadequate exposure to N-DEAQ in pediatric patients.

Both amodiaquine and N-DEAQ were found to be highly bound to plasma proteins, with mean bound fractions of 92 and 85% respectively, which suggest protein binding to be a possible source of inter-patient variability in PK. AGP concentrations varied 3-4 fold in 85 children and 36 adults with uncomplicated falciparum malaria [67, 102]. According to the binding model in paper II, this would translate into a doubling in the N-DEAQ unbound fraction, and as a consequence, in the total N-DEAQ plasma concentrations. There are also time dependent changes in AGP concentrations during malaria infection [67]. Although changes in the free fraction do not alter the unbound drug concentrations for orally administered compounds, it may affect the total concentration as shown for quinine in malaria patients [103]. As the exposure response analysis was based on total N-DEAQ concentrations rather than the unbound concentrations this may explain the relatively weak correlation described.

Concomitant intake of high fat foods or beverages have been suggested to improve the absorption, and thus increase the clinical effect, of both lumefantrine and piperazine [40, 54, 79]. We could not detect a significant impact of milk intake on the PK parameters of lumefantrine. A possible explanation for the discrepancy in the results compared to the previous report by Ashley and colleagues [104] is the fact that our patients were reluctant to drink the milk in almost 50% of administrations. The resulting number of doses actually administered with milk may have been too small to allow the detection of a difference.

We did not identify a difference in exposure to piperazine due to concomitant intake of a relatively low-fat meal. As indicated by the variable extent of food effects in other studies, from a two-fold increase in piperazine exposure with a high-fat meal to the less pronounced increase of 40% with a meal containing less fat [51, 54], it is likely that the effect is highly dependent on fat-content. Given the considerable interindividual variability in exposure, as shown by the 20-fold range in AUC_{0-last} in both fed and fasting subjects there are other factors contributing significantly to differences in piperazine PK.

The logistic effect model used to describe the dichotomous outcome data in Zanzibar is a good tool to evaluate the influence of PK on data typically obtained in studies of malaria treatment, namely day 14 and/or day 28 treatment outcome. However, this non-mechanistic model does not allow for the closer assessment of the effect of PK on parasite dynamics during treatment, such as the slope of the

association between drug concentration and parasite death rate. While the artemisinin derivatives appear less prone to induce resistance compared to other antimalarials, singular reports suggest the occurrence of parasite strains with reduced sensitivity to DHA [105]. Determining and comparing the variability in the association between concentration and effect could be a valuable tool in monitoring parasite susceptibility.

Models describing the *in vivo* dynamics of untreated *P. falciparum* infection have primarily been developed from data in adult, non-immune, patients inoculated with malaria to treat neurosyphilis [59, 106]. The pharmacodynamic model in paper III is based on data from both symptomatic and asymptomatic children in a rural setting in Tanzania. While the influence of the presence of poly-clonal infection, semi-immunity, fever and other clinical symptoms of malaria, on parasite population dynamics *in vivo* needs to be investigated in further studies, some basic model parameters should be translatable from asymptomatic to symptomatic patients. In the proposed model we assumed that the mean transit time through the erythrocytic cycle was equal in both groups and that the rate of development through the parasite stages was the same across patients and asymptomatic carriers. The estimated parameters pertaining to the growth and development of the parasite population, namely the mean transit time through the erythrocytic cycle estimated to 48.5 hours, the mean time to sequestration estimated to 17 hours, and the multiplication factor of parasitemia in patients estimated to 10-12 per erythrocytic cycle all compare well with previous reports [106-109].

The proposed PD model supports the observation of a previous study showing the initial decline in parasitemia to result mainly from the artemether/DHA component during combination treatment with lumefantrine [20]. The addition of an effect of lumefantrine did not significantly improve the model fit. The predicted median parasite clearance time (PCT) was 38 hours which compares well with the observed median PCT of 36 hours. A considerable drawback of the PK-PD study of artemether and lumefantrine was the short study period of only three days. Many treatment failures occur later than three days after treatment, following an earlier parasitological cure. A longer follow up may have provided the possibility to model the risk of these late failures and to accurately model the lumefantrine effect.

The effect of artemether and DHA concentrations on the parasite density in malaria patients was adequately described by the proposed semi-mechanistic model of parasite dynamics. To make full use of the proposed model, however, stage-specific parasite counts should be obtained both prior to, and during, drug treatment.

Conclusion

There was a significant, albeit weak, correlation between the clinical outcome of the combination amodiaquine+artesunate and exposure to N-DEAQ. Amodiaquine and N-DEAQ were both shown to be highly bound to plasma proteins *in vitro*, which may explain the difficulty in establishing a good concentration-effect relationship from total N-DEAQ concentrations. While earlier studies have shown a considerable effect on the exposure to lumefantrine when co-administered with soy milk or food, there was no significant correlation between PK parameters and milk intake in pediatric malaria patients. However, many children refused the milk and the result may have been due to lack of study power rather than a true lack of effect. There was no significant impact on the exposure to piperaquine due to concomitant intake of a relatively low-fat meal. The 20-fold range in exposure in both fed and fasting subjects suggests that there are other factors contributing significantly to interindividual differences in piperaquine PK. The effect of artemether and DHA concentrations on the parasite density in malaria patients was adequately described by the proposed semi-mechanistic model of parasite dynamics, with predicted median parasite clearance time corresponding well with the observed. To make full use of the proposed model, however, stage-specific parasite counts should be obtained both prior to, and during, drug treatment.

Swedish summary

Populärvetenskaplig sammanfattning

Halva jordens befolkning lever under hotet att insjukna i malaria. Flest malaria-relaterade dödsfall sker bland barn i Afrika, söder om Sahara. Ett stort problem vid behandling av malaria är resistensutveckling hos malariaparasiterna. Läkemedel som tidigare haft god effekt mot sjukdomen fungerar inte längre. Sedan 2006 rekommenderar Världshälsoorganisationen att malariabehandling skall bestå av kombinationer av läkemedel, där de ingående substanserna angriper parasiterna på olika sätt.

Syftet med det här arbetet var att studera några av dessa kombinationer för att utröna hur substanserna tas upp, fördelas och sedan utsöndras från kroppen (farmakokinetik), samt vilken effekt de har vid behandling av malaria (farmakodynamik).

I samband med implementeringen av nya behandlingsrekommendationer i Tanzania genomfördes kliniska studier för att karaktärisera farmakokinetiken och farmakodynamiken av läkemedlen hos barn. Sådana studier är viktiga för att visa att den dosering som används och som utvecklats för vuxna patienter, faktiskt fungerar även hos barn. Studierna visade att farmakokinetiken för de använda substanserna (amodiakin/desetylamodiakin, lumefantrin samt artemeter/dihydroartemsinin) liknar farmakokinetiken hos vuxna. Trots detta var blodnivåerna av den aktiva substansen desetylamodiakin relativt låga hos de behandlade barnen. Resultatet kan bero på att många patienter inte följde behandlingsrekommendationerna, men även på att den åldersbaserade dosering som rekommenderas resulterar i för låga doser.

Vissa födoämnen kan påverka hur en medicin tas upp och fördelas i kroppen. Tidigare studier har visat att blodkoncentrationerna av malarialäkemedlen piperakin och lumefantrin blir högre om man tar tabletterna i samband med en måltid eller fet mjölk. Detta har lett till antagandet att effekten av dessa läkemedel skulle kunna förbättras genom kombination med mat eller fett-innehållande dryck. I en undersökning bland malariasjuka barn påvisades dock ingen effekt på upptaget av lumefantrin vid samtidigt intag av ett glas fet mjölk. Många av barnen ville inte dricka upp mjölken vilket resulterade i att relativt få doser faktiskt togs tillsammans med mjölk. Intervention är således sannolikt av ringa kliniskt värde. Detsamma visade sig gälla för intag av piperakin tillsammans med en typisk Vietnamesisk måltid. Den Vietnamesiska dieten innehåller i genomsnitt betydligt mindre fett än

den västerländska, vilket sannolikt är orsaken till skillnaden i effekt på upptaget av piperakin.

I de flesta studier av effekten av malariabehandling tittar man på hur många patienter som tillfrisknat en månad efter behandlingens start. Detta är ett bra mått på effekten av långverkande läkemedel som amodiakin, lumefantrin och piperakin. Det är något sämre då det gäller att studera effekten av snabbverkande preparat som exempelvis artesunat och artemeter. Genom att anpassa en matematisk modell som beskriver parasitens livs-cykel och hur denna påverkas vid läkemedelsbehandling kunde en närmare beskrivning av den tidiga läkemedelseffekten göras.

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