Population Pharmacokinetic Modelling and Simulation of Antimalaria drugs to optimize dosing in neglected populations

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Dedicated to all children at risk of malaria

Preface

This PhD thesis was undertaken at Epidemiology and Public Health department of Swiss Tropical and Public Health Institute and the faculty of Science of University of Basel, Basel, Switzerland in partial fulfillment of the requirements for acquiring the PhD degree in Epidemiology.

The PhD work was financially supported by Swiss TPH and Basel Stadt (9 months sponsorship). The work was carried out in the period from July 2013 to June 2017 in collaboration with Pharmacometric group, division of Clinical Pharmacology, Department of Medicine at the University of Cape Town, South Africa.

The topic of the PhD thesis is population pharmacokinetic modelling and simulation of antimalaria drugs to optimize dosing in neglected populations. The thesis consists of a summary, report and three peer reviewed scientific research papers written during the PhD study.

The work was supervised by Prof. Dr. Melissa Penny (Swiss TPH) and Dr. Paolo Denti (University of Cape Town). Prof. Goonaseelan (Colin) Pillai (Novartis AG) was external examiner and Prof. Marcel Tanner (Swiss TPH) representing the faculty. This PhD committed was chaired by Prof. Dr. Thomas Smith (Swiss TPH)

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ACT	Artemisinin combination therapy
AL	Artemether-lumefantrine
AQ	Amodiaquine
ART	Artemisinin
AS	Artesunate
AS-AQ	Artesunate-amodiaquine
AUC	Area under plasma concentration-time curve
BCTU	Bagamoyo clinical trial unit
BIO	Bioavailability
BLOQ	Below the limit of quantification
BOV	Between occasions variability
BSA	Body surface area
BSV	Between subject variability
BW	Body weight
BWstd	Body weight of standard patient
CCM	Community case management
CHWs	Community health workers
CI	Confidence interval
CL	Clearance
CL_H	Hepatic clearance
CLint	Hepatic intrinsic clearance
CLstd	Clearance of standard patients
Cmax	Maximum concentration
CQ	Chloroquine
CV	Coefficient of variation
CWRES	Conditional weighted residuals distribution
CYP	Cytochrome P450

CYP2C8	Cytochrome P450-2C8
DALYs	Disability-adjusted life years
DE	Disease effect
DEAQ	Desethylamodiaquine
DHA	Dihydroartemisinin
E_H	Hepatic extraction ratio
etc.	*et cetera*
F	Relative bioavailability
FC	Fractional change
FDC	Fixed dose combination
FFM	Fat free mass
F_H	Hepatic first-pass extraction
Fmat	Maturation function
FO	First-order
FOCE	First-order conditional method
fu	Fraction unbound
GFR	Glomerular filtration rates
GMAP	Global malaria action plan
HB	Hemoglobin
HT	Hematocrit
Ht	Height
IHI	Ifakara health institute
IPRED	Individual prediction
IPTp	Intermittent preventive treatment
IQR	Interquartile range
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
i.v	Intravenous

iWRES	Individual weighted residuals
Ka	Rate of absorption
Ke	Rate of elimination
kg	Kilogram
LLOQ	Lower limit of quantification
Ln	Natural logarithm
L/h	Litter per hour
MDG	Millennium development goals
mg	Milligram
MMV	Medicine for Malaria Venture
MS	Mass spectrometry
MTT	Mean transition time
NCA	Non-compartmental analysis
NIMR	National institute for medical research
NONMEM	Nonlinear mixed effect modelling
NPQ	Naphthoquine phosphate
OBS	Observed value
OFV	Objective function value
PBPK	Physiologically-based pharmacokinetic
PD	Pharmacodynamic
pf	Plasmodium falciparum
PK	Pharmacokinetic
PKPD	Population pharmacokinetics pharmacodynamics
PMA	Post menstrual age
PMA_{50}	Postmenstrual age at 50% of adult maturation
PNG	Papua New Guinea
Pop-PK	Population pharmacokinetic
PRED	Population prediction

PsN	Perl-speaks-NONMEM
pv	Plasmodium vivax
Q	Peripheral clearance
Q_H	Hepatic plasma flow
QTc	Corrected QT interval
RBC	Red blood cells
RBM	Roll back malaria
RDT	Rapid diagnostic tests
RR	Risk ratio
TFDA	Tanzania food and drug authority
T_{max}	Time to reach maximum concentration
$t_{1/2}$	Elimination half-life
V	Apparent volume of distribution
VPCs	Visual predictive checks
V_{ss}	Volume of distribution at stead state
WHO	World health organization
WWARN	Worldwide Antimalarial Resistance Network

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Summary

Malaria is a mosquito-borne infectious disease which affects humans and other animals. In 2016 the World Health Organization (WHO) estimated 212 million malaria cases and 429,000 deaths occurred globally in the year 2015. This results in one child dying after every two minutes. Historically, children experience higher burden of the disease due in part to their lower immunity. The prevention of malaria disease relies on vector control. Two forms of vector control are currently available; insecticide-treated mosquito nets and indoor residual spraying. While for treatment, artemisinin combination therapy has been recommended by the WHO.

Children are at risk of suboptimal dosing of antimalarials, with lower drug exposure related to malaria recrudescent in young children. Optimal curative dosing in adults is informed by extensive safety and efficacy clinical trials, but for children, dosing is currently based on extrapolation from adult doses. Often linear relationships between dose and body weight are assumed, which is the same as treating a child as a small adult. However, children differ from adults in regard to their response to drugs, changes in body weight, and changes in other factors which determine pharmacokinetic (PK) and pharmacodynamics (PD) relationships. These factors include delayed maturation of drug-metabolizing enzyme and maturation of renal function, and expression of receptors and protein involved in PD. To address these factors and improve dosing, we developed population pharmacokinetic models for several antimalarials and used simulation to recommend optimal dosing in children for two antimalarials.

Two antimalarial drugs; amodiaquine (AQ) and naphthoquine (NPQ) were investigated in this thesis. Pharmacokinetic data of amodiaquine collected via 8 clinical studies were used, involving both adult and children uncomplicated malaria patients in African countries and in one Asian country. For both amodiaquine and naphthoquine, population pharmacokinetics (pop-PK) of drug concentration-time data were analysed using non-linear mixed effects methods with NONMEM statistical software. Covariates were analysed and the goodness of fit of each model was assessed via statistical tests and diagnostic plots.

Individual parameter estimates from the final pop-PK models were used to simulate day 7 concentration (exposure) and maximum plasma concentration (Cmax) for both antimalarials. The simulated exposure values were compared across different weight bands, defined according to the manufacturer's recommendation and were evaluated for each available tablet strength. For each weight band a new dose was recommended if the median exposure for each weight was less than 80% of median day 7 concentration of the typical patient.

Both desethylamodiaquine and naphthoquine concentration time profiles were best described by three disposition kinetic models (3 compartment). For amodiaquine the kinetics were best described by two disposition kinetics (two compartment). Amodiaquine bioavailability was found to increase with the time (day) after drug administration and clearance maturation with age. Children at birth were determined to have 27% of adult clearance for amodiaquine and 24% for desethylamodiaquine. In addition, children were determined to reach 50% of the weight-adjusted adult clearance values at 3 and 4 months after birth for amodiaquine and desethylamodiaquine and 100% values at about 3 years of age. No covariate was found to be associated with pharmacokinetic parameters of the naphthoquine.

Simulation results indicated that the day 7 concentrations of amodiaquine and naphthoquine in children were on average 25-30% lower than a typical 50 kg patient and hence higher doses are needed compared to current recommended doses. The optimized dosing regimen recommended includes higher doses per kg for younger children. In particular, higher mg/kg doses are recommended in smaller children, consistent with the nonlinear effect of body size on clearance described by allometric scaling. The optimized regimen proposed in this thesis aims to achieve more a balanced exposure across weight bands without any risk of toxicity. They will need to be further assess in light of operational, safety, and clinical studies.

This is the first study to investigate the suboptimal plasma exposures level of amodiaquine and naphthoquine antimalarials and hence confirm the previous findings of other antimalarials that dosing in young children is not adequate when based on allometric scaling alone. Simulation of well calibrated pop-PK, models, indicates that for young children and patients of weight above 60 kg require higher doses than the current manufacturer recommended dose. The optimized dose will ensure similar exposure levels over all patient's weight range and hence reduce the selective pressure of the development of drug resistance.

Chapter One

This chapter provides an overview of the problem of malaria in young children, effort that in place for the control and treatment of malaria disease. It also highlights the problem of the current dosing strategy in young children and provides the way forward in solving the issue of dosing in this population. The chapter ended by stating the objective of this thesis.

1. Introduction

1.1 The Global and African burden of malaria

Malaria is a mosquito-borne infectious disease which affects humans and other animals. The disease is transmitted by an infected mosquito by injecting malaria parasites into vertebrate host's blood. The parasites are micro-organisms that belong to the genus *Plasmodium of which four species (Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale) have been identified to cause malaria in humans* (1)*. Of the four species Plasmodium falciparum has long been recognized as the species causing the majority of severe and fatal malaria cases.* This thesis focuses on treatments for Plasmodium falciparum.

There has been tremendous progress worldwide in the fight against malaria disease. The 2016 malaria report of the World Health Organization (WHO) estimated that global malaria cases declined by 19% in the period 2000-2015 from 262 million to 212 million. This represents a 41% drop in malaria incidence when taking into account population growth (2). Similarly, there was an approximate 50% decline in malaria deaths worldwide in all ages between the years 2000-2015. Nevertheless, despite substantial progress there was a total of 212 million malaria cases and 429,000 deaths estimated to have occurred globally in the year 2015 alone, with 70% of those malaria deaths occurring in children under five years of age. African countries carry the highest burden, with 90-95% of malaria cases and deaths occurring in Africa (2). In 2015 malaria caused 56 million years of health life to be lost worldwide and malaria ranked as among the top four leading cause of lost disability-adjusted life years (DALYs) in Africa, with 50 million future healthy life-years lost in 2015 alone (3).

Large scale roll out of insecticide treated nets (ITNs), indoor residual spraying (IRS) and malaria treatment are largely responsible for this reduction in malaria morbidity and mortality (4), however future successes are challenged. The tendency of malaria parasites to develop multiple strategies to deal with the anti-parasite action of non-artemisinin antimalaria drugs (5, 6) and the emergency of resistance to monotherapies (7) has contributed to this challenging situation. Significantly, the emerging and increasing resistance to artemisinin, a key malaria drug in first-line therapy, raises the risk of wide-scale drug resistance (8, 9). Systems effectiveness for malaria case management is another challenge in most of African countries where the disease is predominant. It is well known improving health and disease treatment should be coupled with stronger health systems. Poor access to malaria treatment, poor compliance with treatment guidelines by the health providers, poor patient adherence to the treatment, and substandard medication (10) are among the factors contributing to the failure in malaria case management. At the same time, suboptimal antimalarial dosing of young

children (11–16) and non-adherence to recommended dosing has resulted in continued disease burden and has potential to further increase the risk of wide-scale drug resistance.

1.2 The Global effort to control malaria

The reduction and control of malaria over the last decades has been achieved via a continous process with many players; individual people, countries and international organizations. In this time, several organizations have set goal(s) aiming for increased control of the disease. In 2000, the United Nations set the Millennium Development Goals (MDGs) with the aim of improving health for all human beings. MDG 6 aimed to "have halted and begun to reverse, by 2015, the incidence of malaria and other major diseases" (17). Three indicators were introduced in order to track the progress of malaria morbidity, mortality and the implementation of intervensions. The indicators were "(i) incidence and death rates associated with malaria, (ii) proportion of children under 5 sleeping under insecticide-treated mosquito nets and (iii) proportion of children under 5 with fever who are treated with appropriate antimalarial drugs" (17). Many countries especially in Sub-Saharan Africa, worked towards achieving this goal and as a result a 48% decline in malaria death and 12% in malaria cases has been observed in African countries from the start of declaration (in 2000) to 2015 (17). In particular, progress for indicator (iii) has been significant as a result of introduction of new combination treatments. However, there is room to improve by ensuring drugs are developed for children and access to treatment is increased towards universal coverage.

The Abuja declaration and its plan of action was another declaration set in the year 2000 with the aim of strengthening the effort towards malaria elimination in African countries (18). African leaders in this summit committed themselves to intensive efforts to halve the malaria mortality in Africa region by the year 2010 (17). Although this goal was not achieved; substantial progress was made with malaria mortality decreasing by 35% in Africa, (17). In 2005, the World Health Assembly set a resolution for malaria control, which required each member of state to establish policies and operational plans in their state that would enable those at risk of, or suffering from, malaria to receive major preventive and curative interventions by 2010 and thus reduce the burden of malaria by at least 50% by 2010 and 75% by 2015 (19).

In 2008 the Roll Back Malaria partnership (RBM) through its global malaria action plan (GMAP) set the objective of reducing malaria deaths to close to zero by 2015 for all preventive deaths (20). Towards achieving this, the GMAP defined two stages of malaria control: 1) *scaling-up for impact* of preventive and therapeutic interventions, and 2) sustaining *control* over time. They additionally identified three areas of research on malaria control and elimination: 1) research and development for new tools, 2) research to inform policy and 3) operational and implementation research (20). Table 1.1 present the list of objectives or targets with their corresponding indicators (21). These targets provided the

direction for the design and framework for monitoring and evaluation of malaria control program. The World health organization currently recommends a package of interventions to control and prevent malaria and its effect during pregnancy (22, 23).

Table 1.1 Roll Back Malaria objectives, targets for 2015 and indicators for measuring progress

GMAP objective or target	Key indicators
Objective 1. **Reduce global malaria deaths to near zero* by end 2015**	Inpatient malaria deaths per 1000 persons per year All-cause under-five mortality rate
Target 1.1 Achieve universal access to case management, in the private sector	% suspected malaria cases that receive a parasitological test % children aged under 5 years with fever in the last two weeks who had a finger/heel stick
Target 1.2 Achieve universal access to management, or appropriate referral, in the private sector	% confirmed malaria cases that receive first-line antimalarial treatment according to national policy
Target 1.3 Achieve universal access to community case management (CCM) of malaria	% receiving first-line treatment among children aged under 5 years with fever in the last 2 weeks who received any antimalarial drugs
Objective 2. **Reduce global malaria cases by 75% by end 2015 (from 2000 levels)**	Confirmed malaria cases (microscopy or RDT) per 1000 persons per year Parasite prevalence: proportion of children aged 6–59 months with malaria infection
Target 2.1 Achieve universal access to and utilization of prevention measures**	% population with access to an ITN within their household % population who slept under an ITN the previous night % population protected by IRS within the last 12 months % households with at least one ITN for every two people and/or sprayed by IRS within the last 12 months
Target 2.2 Sustain universal access to and utilization of preventive measures	% women who received intermittent preventive treatment for malaria during ANC visits during their last pregnancy
Target 2.3 Accelerate development of surveillance systems	% districts reporting monthly number of suspected malaria cases, number of cases receiving a diagnostic test and number of confirmed malaria cases
Objective 3. **Eliminate malaria by end 2015 in 10 new countries (since 2008) and in the WHO European Region**	Number of new countries in which malaria has been eliminated

Adopted from World malaria report 2015 (17)

*In areas where public health facilities are able to provide a parasitological test to all suspected malaria cases, near zero malaria deaths is defined as no more than 1 confirmed malaria death per 100 000 population at risk.

**Universal access to and utilization is defined as every person at risk sleeping under a quality ITN or in a space protected by IRS and every pregnant woman at risk receiving at least one dose of intermittent preventive treatment (IPTp) in settings where IPTp is appropriate.

More recently, the RBM has reviewed its global malaria action plan, with the slogan of "*Action and Investment to defeat Malaria 2016–2030 (AIM) – for a malaria-free world*". AIM targets to reduce malaria cases and deaths by at least 90% by 2030 as compared to 2015. It also aimed to eliminate malaria from at least 35 countries by 2030 from the countries where malaria was transmitted in 2015. The last goal set is to prevent re-establishment of malaria in all countries where malaria has been eliminated (24).

The control of malaria requires a combination of both preventive and curative measures. Preventive measures include use of chemoprophylaxis and attacks on the mosquito vector to stop and prevent transmission from the vector. Vector control includes the use of insecticides against adult mosquitoes, removal or modification of breading sites, larvicides, reducing the contact between people and mosquitoes as well as killing mosquitos by using insecticide treated nets, and use of repellents (25). The use of vector control tools has increased dramatically over the last decades (2) and significantly contributed to the decline of malaria cases (4, 26, 27). Antimalarial drugs are designed to prevent and/or cure the disease. Antimalarials are used to treat and cure suspected or confirmed case of malaria or can be used as preventative treatment in vulnerable population such as pregnant women and infants via intermittent preventive treatment (IPT). Chemoprophylaxis may also be used to prevent malaria infections in travelers who have no immunity and are temporarily visiting malaria endemic countries.

Malaria infections induce incomplete immunity against parasite stages in the human host, and despite being incomplete, the immunity acquisition is over a long time with multiple infections required (28). Malaria vaccines have, and are being considered, as a means to boost immunity especially in very young children who have not developed any natural immunity against malaria infections (28). Malaria vaccines have been in development for decades (29) and most recently a malaria vaccine, the RTS,S/AS01 vaccine (30, 31), completed final Phase III clinical testing with a recommendation from the WHO for further pilot studies to demonstrate mortality effect and feasibility in implementation. This vaccine is only partially efficacious (30, 31), but still offers hope that vaccines may become complementary tools in malaria programs. Future malaria vaccines should be used in conjunction with

existing tools and will not offer a single solution. Thus, development of new vector control measures and antimalarial drugs must continue.

The importance and use of antimalarial drugs have been recognized since the First World War and between 1960 and 1970 quinine was restored to its traditional role as a major antimalarial drug. Quinine is extracted from traditional herbal *Cinchona* alkaloids (25). Four chemical compounds are extracted from the *Cinchona* (Quinine, Quinidine, cinchonine, cinchonidine), however, only quinine and quinidine have been used (32). Chloroquine is another antimalarial drug that has a long history in malaria treatment. Synthesized since 1934 (25, 32), chloroquine belongs to the 4-amonoquinolines group of compounds. Amodiaquine and naphthoquine are among the members of 4-aminoquinolines compound (32, 33). The decline in efficacy for chloroquine (32) and the emergence of *P. falciparum* (34, 35) and *P. vivax* strains resistant to chloroquine (36, 37), necessitated discoveries of newer antimalarial drugs. In 1972 artemisinin was isolated from *qinghao* (the blue-green herb, *Artemisia annua*) by Chinese scientists and was used as an antimalarial treatment from 1974 (38). The most important derivatives of artemisinin are, artesunate, dihydroartemisinin, artemether and artemether with all being potent fast acting and with rapid parasite clearance. However, several studies have reported the emergence of artemisinin resistance in patients with *P. falciparum* (8, 39–43). The emergence of chloroquine (34, 36) and artemisinin (8, 39) resistance when they have been used as monotherapy has led to the recommendation of using drugs in combination.

In 2000 the World health organization endorsed the use of artemisinin combination therapy (44) as the first-line treatment for uncomplicated malaria. Moving from antimalarial monotherapy to artemisinin combination therapy (ACTs) aimed at delaying the appearance of drug resistance to one or both partners in combination. The five ACTs currently recommended by WHO (2013) are artemether+lumefantrine, dihydroatrmisinin+piperaquine, artesunate + sulfadoxine–pyrimethamine (SP), artesunate + mefloquine and artesunate+amodiaquine (45). Currently a new ACT (artemisini+naphthoquine) have been developed and is already used in the market even though it is not on the list of WHO recommended antimalarials. For the purpose of this thesis, antimalarial amodiaquine (given alone or in combination with artesunate) as well as artemisinin-naphthoquine will be discussed.

1.3 Amodiaquine for the treatment of malaria

Structurally amodiaquine (Figure 1.1) is closely related to that of chloroquine (CQ), both being a member of 4-aminoquinolines compounds (32). However, amodiaquine was found to be more active than CQ against some strains of CQ-resistant *P.falciparum* (46). Amodiaquine was removed from the WHO list of recommended antimalaria drugs in developing countries in the late 1980s (47) after have been associated with toxicity (48–50). However, amodiaquine was re-introduced in 1996 (51). More

recently, amodiaquine has been introduced as a partner drug of artesunate in the WHO recommended ACTs (52).

Artesunate-amodiaquine is one of five ACTs currently recommended by WHO and is predominantly used as a second line treatment after the first line treatment artemether-lumefantrine (52). Three different formulations are available for the combination; loose tablets, co-blister pack and as a fixed dose combination. Amodiaquine given as a fixed dose formulation with artesunate was recently shown to be associated with higher efficacy than the other formulations (53). Antimalaria responses are increased by amodiaquine due to it's rapid absorption and contrasting shorter elimination half-life of less than one hour of partner drug artesunate (54, 55). After oral administration, amodiaquine is rapidly absorbed and undergoes extensive metabolism and converted to its biologically active metabolite, desethylamodiaquine, in the liver by the cytochrome P450 mainly by the hepatic enzyme CYP2C8 (47, 56). Amodiaquine is considered a prodrug as it is quickly cleared from the blood and its active metabolite attains high concentration for a long time (long elimination half-life) (56, 57). Amodiaquine and its metabolites are over 90% bound to plasma protein (57). The therapeutic dose for amodiaquine is 10 mg/kg/day for three days with a lower dose of amodiaquine was associated with 3.5-fold higher risk of recrudescence (53).

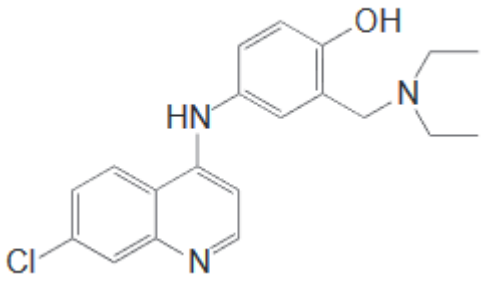

Figure 1.1 Chemical structure of amodiaquine hydrochloride

1.4 Artemisinin and Naphthoquine (ARCO) for the treatment of malaria

Artemisinin-naphthoquine phosphate is a new ACT recently introduced on the market. It is registered under the trade name of ARCO (Kunming pharmaceuticals, Kunming, China). Artemisinin-naphthoquine phosphate (ARCO) is given as a single dose, unlike others ACTs, and thus this schedule results in single dosing of artemisinin contrary to the WHO recommendation for artemisinin in combinations of three days (58). This formulation is intended to improve patient adherence (59). However, there is evidence that multi-dose ARCO will result in higher efficacy (60) furthermore resistance to artemisinin (ART) argues against single doses of ART.

ART has a fast acting parasiticidal action (61, 62), but when used as monotherapy it results in high recrudescent rates due to its very short circulating half-life, (63) and hence has risk of developing drug resistance (8, 9). Naphthoquine phosphate, (NPQ), a partner drug, on the other hand, has a longer half-life (64, 65) and has been reported to have higher cure rates compared to artesunate (66), and slower onset of parasite killing (67). Combining artemisinin and naphthoquine provides an advantage over single drugs by overcoming those individual weaknesses. The safety and efficacy profile of ARCO has been evaluated via several studies and shows comparable efficacy and safety profile to existing antimalarials (67).

Due to the short circulating half-life of artemisinin (63), the main antimalarial activity of ARCO is governed by naphthoquine . Naphthoquine like amodiaquine, belongs to the 4-aminoquiniline family of compounds (33), and it is only available in its phosphate form (64). Figure 1.2 shows the structural form of naphthoquine. After oral administration, naphthoquine is widely distributed to the liver, kidney and the lung in that order and the main route of excretion is in urine (64). The mechanism of action for naphthoquine is not well established, however, it is expected to resemble that of other 4-aminoquiniline compounds (33).

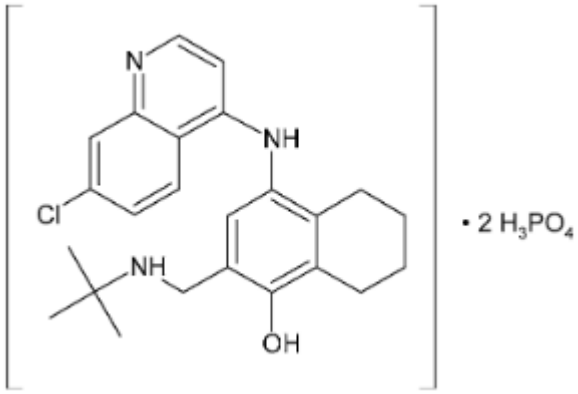

Figure 1.2 Chemical structure or naphthoquine phosphate

1.5 Dosing in children

Most antimalarials available on the market were initially developed for adults and the decisions for dosing regimens for children were mostly based on assumptions of a linear relationship between an adult's drug dose and child's body weight, surface area, or age (68–70). The mathematical formulas (68) in scaling adult doses to children normally use body weight (BW) and body surface area (BSA) (equation 1 and equation 2 respectively).

$$Dose_P = Dose_A . \frac{BW_P}{BW_A} \quad (1)$$

$$Dose_P = Dose_A . \frac{BSA_P}{BSA_A} \quad (2)$$

Where $Dose_P$ = dose in pediatric, $Dose_A$ = dose in adults, BW_P and BSA_P are pediatric values and BW_A and BSA_A are adults values for these variables respectively.

An alternative model used to scale pharmacokinetic parameters between adults and children is the allometric power models (68), with general equation:

$$Y = a . BW^b \quad (3)$$

Where Y is the biological characteristic to be predicted, a and b are empirically derived constant and exponent, respectively.

For drug combinations, the dosing regimen of each drug is generally identified through clinical trials involving only adults and the dosage ratio is then fixed, and afterwards used as the basis to determine treatment regimen in other populations (71), including children. The assumption imposed is that the same ratio of the components of combination drug that leads to therapeutic exposure levels in adults will yield comparable exposure levels in children and be as effective and safe (71). For example with ARCO, which is the combination of Artemisinin and Naphthoquine, the ratio between the two compounds is the same between adults and children (2.5:1, artemisinin : naphthoquine) though the dose for children is lower, based on their body weight (67). The same applies to Coartem which comprises the active substances Artemether and lumefantrine. Children receive lower doses compared to adults but, the ratio between the two active compounds is the same (1:6) for both adults and children (72). This is equivalent to taking adult and cutting them into pieces and considering the pieces as equivalent to children or treating a child as a small adult.

“The aphorism ‘children are not small adults’ holds true not only for the selection of suitable drugs and dosages for use in children but also for their susceptibility to adverse drug reactions” (73, 74). Children differ from adults in regard to their response to foreign substance/chemicals, including drugs. Apart from changes in body weight in children there are other factors which determine the changes in pharmacokinetic (PK) and pharmacodynamic (PD) relationship. These factors include maturation of enzymes in pathways involved in the PK, function and expression of receptors, and proteins involved in PD (75), variability in regional blood flow, organ perfusion, permeability of cell membranes, delayed maturation of drug-metabolizing enzyme and maturation of renal function (76).

Considerable PK and PD changes occur at early stages of childhood and develop from preterm infants, towards term, as infants maturing through the first few years of life, and as children approaching adolescence. Thus, the pediatric population alone comprises a range of dissimilar physiologies and different subgroups by age (77). The variation in body composition and the differences in function of

the livers among neonates, infants, children, and adults, are likely to be the main sources of pharmacokinetic differences among them (76). Unlike in adults, drugs in children depend on the developmental stage (organ maturation) as well as individual specific growth (78) and hence it is not applicable to assume linear relationships apply between dose and body weight or age. However, researchers have been forced to make these simplifying approximations by the difficulty in conducting early phase clinical trials in children whereby PK and PD assessments of the drugs could be done.

Following different physiologic stages that children pass through over their entire childhood life, it is obvious that a single extrapolation model will not be appropriate to describe the children's PK. Mahmood (2006) used body weight of children and compared six different methods to predict drug clearance in children from adults, and he found that no single method is suitable for all drugs or for all age groups (79). Different scaling factors need to be applied for different age or weight groups and different drugs (68), however, this is not done in practice. Ethical issues and practical challenges (80) considerations in implementing pediatric trials have led to dose finding in children to be based on extrapolations from adults dose.

1.6 Issues concerning the current dose recommendation in children

The current dosing practice for children (68) raises the concern that recommended doses may be inappropriate for some subgroups of patients, such as young and malnourished children or pregnant women, as a result of PK differences in these vulnerable groups. Barnes, K. I. et al (2006) (81) found that the current dose of Sulfadoxine-pyrimethamine in young children is not adequate and the risk of treatment failure was doubled in children aged 2 to 5 years, when compared with all other age categories combined (risk ratio [RR], 2.1 [95% CI, 1.31-3.38]; p= 0 .0026). Sub-therapeutic drug levels resulting from under dosing contribute to poorer responses to treatment and hence increase the emergence and spread of antimalaria drug resistance (16).

The assumptions that adult and children have similar disease progression and response to interventions, as well as similar exposure-response relationships for drugs (82), may not necessarily be true for malaria. Children have historically suffered more malaria morbidity compared to adults due to their lower immunity against malaria, as they have not had a chance to build their immune system with previous exposure compared to adults in an endemic area (28). Moreover, disease severity is generally worse in children, with higher parasite count and lower hemoglobin. Several PK studies have shown children have received lower exposure levels of drugs compared to adults (11–16) when regimens are determined via extrapolation from adult dosing (for example piperaquine). This means children receive lower doses compared to what they should receive or the response of the drug treatment on parasites (efficacy) is not similar to adults. As a consequence, several previous

studies have evaluated the current dose recommendation and proposed new pediatric doses based on modeling and simulation, e.g. piperaquine (15, 83, 84).

1.7 Modelling and simulation in aiding dose regimen selection in children

In 2008 the European medicines agency organized a workshop that provided a guideline on how modelling and simulation can be used to aid drug development in pediatric (85). "A dosing regimen in children that ensures exposure level comparable to that in adults represents a much safer and effective approach"(71). Modeling and simulation-based approaches have been proposed to support pediatric drug development in order to better in efficiently design and analyze clinical studies (86). Bellanti. F & Pasqua O.D (2011) have shown the use of modelling and simulation is an essential tool in assessing the impact of different regimens and in assessing the differences between populations on the safety and efficacy profiles of a drug and also allow the individualization (group specific, according to age or weight) of drug therapies in children (87). Modelling and simulations approaches can use detailed information on PK, and potentially PD of a drug, to define safe and effective dosing regimens throughout pediatric age ranges.

Population PK and PD modelling has gained in popularity due to the availability of advanced statistical methodology and software. Using nonlinear mixed effects models with the help of well-developed software (88, 89) can not only evaluate pharmacological data on a population level but, importantly, simultaneously also estimate the between and within subject variability (90). With such advanced statistical tools, once characterized population PK and PD models can be used to develop rational dosing schemes by predicting concentration profiles (and toxicity) and efficacy for doses outside those tested and for evaluating the appropriateness of doses in high risk groups. Identifying covariates that affect the PK profiles are also important to define appropriate doses based on the characteristics of individual child (80). Population PK can also be used to derive and compare secondary PK parameters summarizing exposure, such as area under plasma concentration-time curve or day 7 concentration.
In order to define safe and effective dosing regimens in children, detailed information on both PK and PD are need. And then simulations can be undertaken to assess critical scenarios that cannot be generated in real-life studies, such as overdosing (91).
Several studies have demonstrated use of PK and PD modelling to improve dosing recommendations. Bergstrand M *et al*. 2014 (15) applied modeling and simulation to improve dosing for piperaquine. They found that, compared to the manufacture's dose recommendation, using the new recommended dose the incidence of malaria in small children (8 to 12 kg) in a 1-year period could be reduced by 70%. Hendriksen ICE *et al*. 2013 (14) used population PK models to propose optimal doses for artesunate in severe malaria children receiving intramuscular artesunate. Population PK modelling and simulation was also used to select oral pediatric dose of penciclovir and define optimal sampling time and

sampling windows for three different age groups of children (92). Zaloumis *et al*. 2014 applied population PK model to modify dose for children receiving artesunate given intravenously (93). These examples provide proof that the use of modelling and simulation provides a means to circumvent the existing difficulties in developing medicinal products in pediatric populations.

1.8 Objectives and outline

The main aim of this thesis is to improve curative dosing of antimalarial drugs in neglected populations, in particular children, and propose dosing regimens that result in comparable exposure levels between adults and children. This is accomplished via three objectives:

i. To use population PK modelling and simulation to evaluate dose-exposure relationships in children with acute, uncomplicated P. falciparum malaria, after receiving fixed-dose combination therapy of ACTs based on current recommendations.

ii. To evaluate in children who received ACTs based on weight or age, whether they achieve comparable exposure levels of active moieties to those of adults.

iii. To define new dosing recommendations using modelling and simulation for children receiving antimalarial combination therapy.

The analysis accounts for many of the factors discussed above in regards differences between children and adults and metabolism of antimalarials.

The organization of the thesis is as follows: in Chapter 2 the clinical studies used throughout the thesis is presented, along with the general pharmacokinetic modelling and statistical methodology. In Chapter 3, population pharmacokinetic modelling of amodiaquine (using venous blood samples) is presented, with analysis undertaken by pooling data from 6 different studies, with the aim to optimize doses in children. While, in chapter 4 a population pharmacokinetic analysis of amodiaquine from capillary whole blood samples is presented. In Chapter 5 the population pharmacokinetics of naphthoquine is assessed via compartmental modelling and exposure between adult and children compared for single and multiple dose regimens. Finally, an overall discussion and conclusion is presented in Chapter 6.

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Chapter Two

This chapter describes general methodology used to model Pharmacokinetic data and methods that applied during analysis for this thesis.

2. Methodology

The specific methodology used in each paper is given in their respective chapters (Chapter 3, 4, and 5), with only necessary information provided so as to align with journal requirements. For coherence, this chapter provides additional detailed information on the pharmacokinetic (PK) data and clinical studies, along with the modelling methods used throughout.

2.1 Clinical studies and data

The analysis in this thesis uses data collected via 8 clinical studies involving both adult and children uncomplicated malaria patients in African countries and one country in Asia. Primarily the data include PK data of the two main antimalarial drugs considered in this research, namely amodiaquine and naphthoquine. In addition, the data include disease or patient characteristics details, such as parasitemia, hemoglobin, age, weight, etc. A detailed description of each study and drug is presented below.

2.1.1 Amodiaquine study (Chapters 3 & 4)

Data were retrieved from the Worldwide Antimalaria Resistance Network (WWARN) database. WWARN is a global platform that provides the malaria community with a reliable data collection platform for antimalarial drugs and provides evidence for policy makers and drug developers to optimize the therapeutic life of antimalarial medicines, thus supporting decisions aiding the fight against antimalarial drug resistance (1). The database included dosing information, drug concentrations, symptoms, parasitaemia, clinical and laboratory data collected from uncomplicated malaria patients administered with either amodiaquine monotherapy or artesunate-amodiaquine combination therapy. Amodiaquine (AQ) was given daily for 3 days alone or in combination with artesunate. Different matrices were used for blood sampling across the studies and the number of samples collected varied by study. Detailed information on how the subjects were dosed, followed up, how the samples were collected, stored, quantified, and analyzed, can be found in chapter 3 and 4 of this thesis and from the respective studies (2–7).

2.1.2 Artemisinin Naphthoquine (ARCO) study (Chapter 5)

Data of this study was collected and provided by Medicine for Malaria Venture (MMV) and the Ifakara health institute (IHI). ARCO has been used in Tanzania for the treatment of uncomplicated malaria, but its safety, tolerability and efficacy has not been previously assessed in this population. The study was undertaken to confirm its safety, tolerability and efficacy and to also determine the PK of the drug in Tanzanian population. Drug exposure was assessed to evaluate if the current dosing in children results in similar exposure levels to adults and thus explore alternative optimal dosage regimens via simulation (Chapter 5).

Naphthoquine (NPQ) was given with artemisinin (ART) as single fixed dose combination. ARCO tablets contain 125 mg artemisinin and 50 mg of naphthoquine. The adult total dose given was 1000 mg of artemisinin and 400 mg of naphthoquine (8 tablets) and for children the dose was derived according to their body weight (20 mg ART + 8 mg NPQ per kg body weight). Blood samples (3ml) were collected from each patient to obtain measurements of artemisinin and naphthoquine plasma concentrations. For artemisinin, an average of 6 venous samples were collected per patient with a minimum of 6 samples and maximum of 6. For naphthoquine on the other hand, the average number of samples collected was 13 per patient with a minimum of 9 and maximum of 13. For artemisinin the samples were collected 30 minutes before dose, and thereafter at 1, 2, 4, 8, 12 and 18 hours after dose. For naphthoquine, the samples were collected 30 minutes before dose (pre-dosing) and thereafter (post dosing) at 1, 2, 4, 8, 12 and 18 hours and then on days 4, 7, 14, 21, 28 and 42. Plasma was separated from whole blood aliquot into cry vials and stored at Bagamoyo clinical trial unit (BCTU) at -80°C and then transferred to Swiss BioQuant, Reinach, Switzerland for quantification.

Both artemisinin and naphthoquine plasma concentration was quantified by reverse phase chromatography followed by detection with triple-stage quadrupole MS/MS in the selected reaction monitoring mode.

2.2 Study area and study subjects

2.2.1 Amodiaquine study (Chapters 3 & 4)

Data on amodiaquine came from 7 different clinical trials in 5 different countries; these countries are: Burkina Faso, Ghana, Kenya, Myanmar, and Uganda, (2–8). Two studies (one study cohort) were undertaken in Myanmar at the Thai- Myanmar border, and comprised the same women with *vivax* malaria (women were sampled twice, once with malaria in pregnancy and once with post-partum malaria) (3, 4). Two studies were from Ghana, one involved children aged 6 months to 14 years (2) and the other involved patients aged 1 months to 60 years (8). One further study in adult malaria patients was from Kenya (6). Two further studies included children, one in Uganda (5) and another in Burkina-Faso (7). Except for the study in Thailand, all studies involved patients with *P. falciparum* malaria. The studies covered a range of transmission and ecological settings with *falciparum* and *vivax*.

2.2.2 Artemisinin Naphthoquine study (Chapter 5)

Only one clinical trial was available for analysis of Artemisinin-Naphthoquine. The study was undertaken in the newly established clinical trial facility, BCTU located at the Kingani a branch of Ifakara health institute in Bagamoyo, Tanzania. Patients with uncomplicated *P. falciparum* malaria aged 6 years to 55 years were eligible for enrolment in this trial. In this study site, malaria transmission reaches peaks during the rainy seasons in May to July (heavy rains) and December to January (short rains). Of the four malaria species, *Plasmodium falciparum* is the predominant malaria species found

in the area (9). Malaria prevalence in children under five in Bagamoyo was estimated to be 15% in 2015-16 from malaria surveys (10).

2.3 Methods

This section provides a general methodology used in the subsequent research chapters, with specific methodology given in each chapter.

2.3.1 Amodiaquine study (Chapter 3 & 4)

Amodiaquine (AQ) was assumed to be completely metabolized to desethylamodiaquine (11). The subsequent amount of desethylamodiaquine (DEAQ) following amodiaquine administration was derived using the molecular weight of amodiaquine, the molecular weight of desethylamodiaquine, and the amount of amodiaquine received (namely the amount of DEAQ = dose of AQ x 327.81 g/mol [molecular weight of DEAQ]/355.86 g/mol [molecular weight of AQ]). The two active compounds were modelled simultaneously. For studies with partially missing information (on dosing date and time), the recommended dosing schedule was assumed to be followed, with 3 doses taken consecutively every 24 hours. Individuals for which no dosing information was received were dropped from the analysis. Individual PK profiles were inspected and if any inconsistency between two high measurements was observed an error was assumed and thus the data was omitted. Amodiaquine tablets are formulated as amodiaquine hydrochloride (amodiaquine salt), thus the salt was first converted to the corresponding base (amodiaquine base), before performing population pharmacokinetic analysis.

2.3.2 Artemisinin Naphthoquine study (Chapter 5)

Because there was no evidence of interaction between artemisinin and naphthoquine, the two drugs were modelled separately. Naphthoquine phosphate was first converted to its corresponding base before modeling was undertaken. Individual profiles of concentration- time plots were investigated and patients and or data points that showed error were further investigated and confirmed with the investigator at IHI before any decision to omit them from further analysis.

2.3.3 Modelling pharmacokinetic data

In general, PK data are obtained following administration of the drug under investigation to clinical patients or healthy volunteers. Normally blood is drawn from a subject following a certain schedule. Whole blood or plasma samples are used for drug concentration quantification. The primary PK data composes the drug concentration, time the sample was drawn, and the dose given.

When analyzing PK data, one generally employs either non-compartmental analysis (NCA) or compartmental analysis techniques, or both. Decision to prefer one methodology over the other depends on what is required from the analysis. If the primary requirement is to determine the level of exposure such as area under plasma concentration-time curve (AUC) and some of the drug's

associated PK parameters (e.g. maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), elimination half-life ($t_{1/2}$), etc.) following administration of a drug, then NCA can be used. If on the other hand, one needs to explain the heterogeneity in the population on the PK parameters, or if the purpose is to predict some feature, then pop-PK (compartmental) modelling is desired.

Non-compartmental analysis

Non-compartmental analysis summarizes the concentration-time profile via a number of summary measures, such as an AUC, total clearance, volume of distribution at steady state (V_{ss}), half-life of elimination ($t_{1/2}$), etc. The main advantage of NCA is that no specific assumption on a specific absorption/elimination or on the type of compartmental model is made (12). This means NCA calculates secondary PK parameters on exposure from observed data. The other advantage is the shorter running time compared to compartmental analysis. Because non-compartmental analysis has been discussed extensively in the literature, and since it was not implemented in this thesis, we will not discuss in detail here and the reader is referred to (12–15) for more details.

Compartmental pharmacokinetic model

Compartmental modelling of PK data assumes the body is built with a number of compartments on which the drug is stored. The compartments are mathematical representations of biological systems of the human being and are not necessarily meant to represent physiology or anatomy specifically (14). Compartmental modelling relies on three fundamental assumptions: (i) the drug is distributed rapidly and homogeneously in a compartment (ii) tissues with similar characteristics may be merged (iii) drug disposition in the body follows first-order processes (15). A compartment is defined as a group of tissues that acts kinetically in a homogenously distinct manner. It is said to be open if it "leaks" to the environment, otherwise it is closed. A multi-compartmental model consists of at least two compartments interconnected to each other so that material movement can occur among some or all of them (14). In PK research, one-, two-, and three-compartmental models have been intensively used.

Population pharmacokinetic model

Population pharmacokinetic (pop-PK) studies the sources and correlates of variability in drug concentrations in the target population (16). PK parameters can vary from individual to individual due to the difference in their demographic, physiological, genetic, and environmental factors, as well as disease condition. Using pop-PK modelling we can therefore assess the extent of the variability and identify the factors contributing to such variability through identification of predictive covariates in the specified population (17). Three components, as depicted in Figure 2.1, are blocks considered in the pop-PK modelling. The first component of the pop-PK model is the structural model, the second being stochastic model and the covariate model as the last.

The structural part of the pop-PK model describes the general makeup of the PK model which is shared among all individuals in a population. This describes if the drug at a population level follows either one-; two-; or three- compartment kinetics. PK parameters describing the drug in the body such as absorption rate, clearance, volume of distribution, elimination, etc, are derived at this stage. Any covariate which is known a-priori to affect PK parameter(s), such as body weight on clearance and volume of distribution (18) can be also included at this level. The PK parameters and the known covariates affecting the PK parameters are modelled as fixed effect parameters.

Stochastic sub-models describe the variability of the population parameter in the structural model. At this sub-model, it captures how clearance of one subject differs from others (between subject variability) or how bioavailability differs between first drug intakes to the second drug intake (between occasions variability). Between subject variability (BSV) refers to the variance of a parameter across different individuals in the population. BSV can be incorporated into the model as an equation, namely

$$\theta_i = \theta_\mu . exp(\eta_i) \qquad \text{Equation 4}$$

Between occasions variability (BOV) may result from taking some food between doses or disease severity etc. (19). BOV is included in the model according to the equation, namely,

$$\theta_i = \begin{cases} \theta_\mu . \exp(\eta_i + \eta_1 OCC_1) \;\; if \;\; Occasion\; 1 \\ \theta_\mu . \exp(\eta_i + \eta_2 OCC_2) \;\; if \;\; Occasion\; 2 \\ \vdots \\ \theta_\mu . \exp(\eta_i + \eta_0 OCC_0) \;\; if \;\; Occasion\; 0 \end{cases} \qquad \text{Equation 5}$$

Here the Occasion 0 is considered as the reference,θ_i is the model parameter for the ith subject, θ_μ is the population mean and η are assumed to be normally distributed with mean zero and variance of ω^2. The variance of η is occasion specific.

To ensure physiologically meaningful PK model parameters, they must be constrained to be greater than zero. To account for that a log-normal distribution is assumed for BSV and BOV parameters (19). The variance estimate (ω^2) is the variance in the log-domain, and therefore this variance is converted to a coefficient of variation (CV) in the original scale (Equation 6) of the parameter:

$$CV(\%) = \sqrt{\exp(\omega^2) - 1}\ \text{X}\ 100 \qquad \text{Equation 6}$$

Another variability is the within subject variability (residual variability), which are unexplained variability in the observed data after controlling for other sources of variability. It includes the error due to model misspecification, assay error, error in drug dose, error in time measurement, etc. Residual variability is modelled using either additive or proportional error model or combining both. These are generally referred to as a random effect (19). After identifying an acceptable structural and stochastic sub-model, the influence of covariates is then explored.

A covariate is a specific individual characteristic that may influence the pharmacokinetics of a drug, these include age, sex, weight, hematologic value, clinical chemistry value, renal disease, liver disease, disease stage etc. (19). In the covariate sub-model, effect of covariates on the PK parameters are investigated. Covariates are primarily selected if they have a physiological meaning on the parameter of interest. However, plots of parameter variability against the covariate (continuous or categorical) play important roles on covariate screening. Depending on the nature of the covariate to be included, several forms of covariate relationships can be investigated. For continuous covariates; linear, exponential, power, piece-wise linear and non-linear can be considered. These can be represented mathematically as:

$$CL = \theta_1 + \theta_2 . Age$$ Equation 7a. Linear covariate model

$$CL = \theta_1 \exp(\theta_2 . Age)$$ Equation 7b. Exponential covariate model

$$CL = \theta_1 . Age^{\theta_2}$$ Equation 7c. Power covariate model

Where: θ_1 is the clearance (CL) when age is zero and θ_2 is the change in CL per unit increase in age for linear covariate model and is the change of ln(CL) per unit increase in age for exponential and power covariate models. These can be incorporated into the model as in equation 7 (e.g. using clearance (CL) as model parameter and age as continuous covariate).

For categorical covariates, additive proportional models can be used as in equation 8 (e.g. using CL as a model parameter and sex as a categorical covariate):

$$CL = \theta_1 + \theta_2 . Sex$$ Equation 8a

Gender can be coded as 1 for male and 0 for female, and then the model can be represented as:

$$CL = \begin{cases} \theta_1 + \theta_2 \; if \; male \\ \theta_1 \quad\quad if \; female \end{cases}$$ Equation 8b

Covariates are added one by one to the basic model using a forward-selection procedure with inclusion criteria p-value of < 0.05 and then backward elimination approach with p-value of < 0.01 or 0.001 applied to retain the covariate (19) in the final model.

In a pop-PK model, data from different phases of the clinical trial or different study protocols, implementing rich or sparse sampling, can be pooled together to estimate pop-PK parameters. This allows estimation of pop-PK parameters more precisely. It also allows assessment of factors affecting the drug under investigation in more accurate ways (20). Pop-PK was used in this thesis to enable us to not only describe and understand, but more importantly to predict, the drug exposure to different weight groups. This was achieved using non-linear mixed effect modelling implemented in Nonlinear Mixed Effect Modelling software (21, 22).

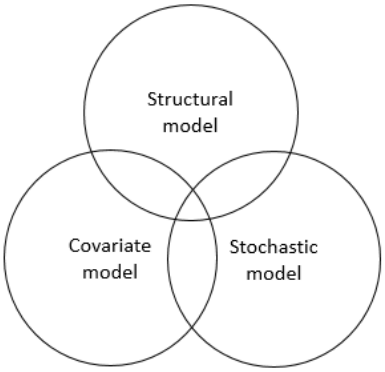

Figure 2.1 Population pharmacokinetic model components: The structural model describes the profile of drug concentration over time for a typical subject, this is common for all subjects (e.g. One-, or two-, or three-compartment). The stochastic model describes the random variability within and between subjects. The covariate model describes the relationship between the structural model parameters and the subject characteristics.

Non-linear mixed effect models

Non-linear mixed effect modelling involves simultaneous analysis of data obtained from all individuals. The parameters of the structural model of the pop-PK are modelled as fixed effect parameters which are the same for all individuals. Further, the approach accounts for different sources of variability such as within subject, between subject variability and between study occasions variability and allows the possibility to separate them from residual variability. For pop-PK models, the drug concentration is the dependent variable and the independent variables are dose and time.

Average pop-PK 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 (23), thereby overcoming important limitations such as ethical and practical limitations in pediatric research (24). Other advantages of non-linear mixed modelling include that no particular structural sampling time schedule or regular sampling time is required for the data and that data may have both subjects with sparse and rich samples (19).

Several software packages have been developed for the analysis of nonlinear mixed effect model (25, 26). However, a nonlinear mixed effect modeling software NONMEM remains the most popular software for pop-PK data analysis in both industry and regulatory board (27). Despite being the current industry standard, NONMEM has some challenges. These include the need to combine with other software for model tracking and diagnostic plots. It is not possible to edit underlying fitting methods, and licenses are expensive (although academic licenses are available), and overall it requires significant training and experience to use appropriately as many of the error messages are difficult to interpret and assess for level of importance.

Estimation methods used for fitting pop-PK models to data are generally based on maximum likelihood principle. Several methods have been proposed for estimating pop-PK parameters (26, 27). Nonlinear

mixed effect model methods establish parameter estimates by minimizing an objective function whose arguments are the parameters of the model (28). First-order conditional estimation methods, as used in this thesis, enables an interaction between within subject and between subject variability, as in proportion residual error model, to be accounted for in the estimation of the likelihood (19).

The objective function value (OFV) as computed by the NONMEM program is calculated as "(–2)* log of the likelihood"(19, 25), thus a lower OFV corresponds to a greater likelihood that the model gives a better fit to the data. Similar to simple linear regression, the likelihood is equal to the sum of square deviations between the predictions and the observations from the model. For nested models, where one is simplified by posing a certain criteria on the other, for example one model with a covariate and one without a covariate, the difference in OFV (log-likelihood ratios) is assumed to be asymptotically chi-square distributed with the degree of freedom equal to the difference in the number of parameters between the models. For 5% level of significance, a decreased OFV of at least 3.84 is considered statistically significant (19).

2.3.4 Data analysis and pharmacokinetic modeling

In this thesis, preparation of data for the pop-PK analysis in NONMEM and calculation of summary statistics of demographics, vital and laboratory parameters, was completed using Stata (version 13, College Station, Texas, USA).

The population pharmacokinetics of venous plasma/capillary blood concentration-time data were analyzed using non-linear mixed effects methods with NONMEM (linear mixed-effects modeling, Icon Development Solutions, Ellicott City, MD) version 7.3. The first-order conditional estimation method (FOCE) (29) with interaction was used for estimation of the population parameters.

One-, two- or three-compartments with first order absorption models, either with lag times or transit compartments (30), were tested. Prior information on the rate of absorption (K_a) and the uncertainty around it was used to avoid unstable estimates in which the rate of absorption is slower than the rate of elimination (flip-flop kinetics). The OFV was used to discriminate between two nested models.

Unexplained residual variability was modelled using a combined additive and proportional error model. Both additive and proportional error models were introduced at the beginning of model development to see if one of the residual variance components is near zero and hence could be dropped from the model.

Interindividual variability terms were introduced after each step of the structural model development and were removed if not supported by the data. A log-normal distribution for between subjects and between occasions in the parameters was assumed as described by equation $\theta_i = \theta \times \exp\left(\mathrm{n}_{\mathrm{i},\theta}\right)$. Where θ_i is the individual parameter, θ is the typical (median) parameter value of the population and

$\eta_{i,\theta}$ is the between subject/occasion variability which assumed to be normally distributed with zero mean and variance ω^2. Between occasions variability was assumed to affect only the absorption rate constant (Ka) and the delay absorption parameter mean transition time (MTT) or the lag time.

Values below the limit of quantification were handled via several approaches: removing the values, imputing half of the lower limit of quantification for the first value in a consecutive series (M6 method in Beal, 31), or a likelihood-based approach (M3 method in Beal, 31). In brief, for the M3 method data below the limit of quantification (BLOQ) are retained and handled as censored observations. The likelihood of all data above the limit of quantification is maximized with respect to the model parameters, and for a BLOQ observation the likelihood is taken to be that the observation is certainly BLOQ. On the other hand, for the M6 method each BLOQ observation is replaced with half lower limit of quantification, with the exception of consecutive BLOQ observation, where all BLOQ observations after the first in a series are discarded. This method assumes that consecutive BLOQ observations reflect true concentrations that are decreasing (31). All data below limit of quantification values were retained for simulation-based diagnostics such as visual predictive checks (VPCs).

Allometric scaling

Allometric scaling using body weight (BW) on clearance and volume of distribution parameters was introduced at the beginning of base model development in each of the chapters. All clearances were scaled using an allometric power of ¾ (Equation 9) and power of 1 (Equation 10) for all volume of distribution parameters (18).

$$F_{size} = \left(\frac{BW}{BW_{std}}\right)^{3/4} \quad (9)$$

$$F_{size} = \left(\frac{BW}{BW_{std}}\right)^{1} \quad (10)$$

Where: WTstd is the weight of standard subject

Fat free mass (Equation 11) (32) was also investigated in place of body weight and the equation which resulted in the minimum value of the objective function (OFV) was chosen.

$$FFM = \frac{WHS_{max} \,.\, Ht^2 . BW}{Ht^2 \,.WHS_{50} + BW} \quad (11)$$

Where: FFM is fat free mass, Ht is height in meter, BW is the observed weight of individual, WHSmax is 42.92 and WHS50 is 30.93 in men and 37.99 and 35.98, respectively, in women.

Maturation effect

Individual clearance estimates were normalized through an age-related maturation function (F_{mat}). The sigmoid hyperbolic or Hill model (18, 32, 34) was used to describe this maturation process (Equation 12). The sigmoid hyperbolic model describes a gradual maturation process at early age, with more rapid maturation occurring at a later age and finally reaching values of an adult (Figure 2.2). Prior information on maturation was used to constrain the parameters of the maturation function to physiological plausible values comparable to those of other drugs (33, 34–38). Maturation of enzymes of the same family to the enzymes responsible for AQ metabolism (39) were used to obtain an estimate of the time until 50% maturation (PMA_{50}), and the estimate of the shape parameter (hill coefficient).

$$F_{mat} = \frac{PMA^{Hill}}{PMA^{Hill} + PMA_{50}^{Hill}} \quad (12)$$

Where: PMA is postmenstrual age (normally in weeks)

While weight describes the effect of size via allometric scaling on clearance, age describes the maturation process of the body during both intrauterine and extrauterine life (18). This developmental stage is normally measured by time and age used in equation 12 and represents the measure of time. The predicted value of clearance (CL) can then be obtained by combining both the effect of size (based on allometric scaling) and developmental process (based on maturation), namely:

$$CL = CL_{std} \,.\, F_{size} \,.\, F_{mat} \quad (13)$$

Where: CLstd is the clearance of standard (typical) subject

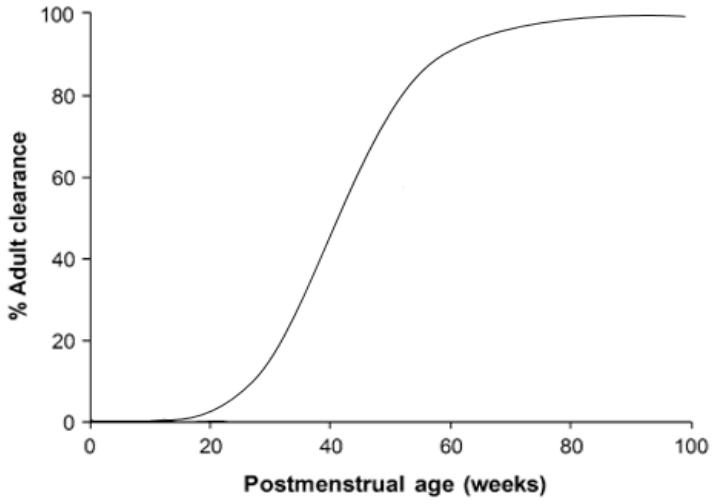

Figure 2.2 Schematic presentation of maturation function

Covariate effects

The relationships between model parameters and the baseline covariates age, parasitemia, hemoglobin, haematocrit, drug formulation (whether amodiaquine was administered alone, or with artesunate, either as loose formulations or a fixed dose combination tablet), sex and fever and other patients' characteristics were evaluated through inspection of scatter plots (continuous covariates) and box plots (categorical covariates). Missing hemoglobin (HB) was derived from hematocrit (HT) value using the equation below (40).

$$HB = \frac{HT - 5.62}{2.60} \tag{14}$$

The formula was first validated by calculating the correlation between actual hemoglobin and hematocrit values from the studies and subsequently hemoglobin was assumed missing and derived based on Equation 14 and correlation was re-calculated Figure 2.3. Covariate selection was based on changes to objective function values (OFV), namely, a drop of OFV > 3.84 was considered significant (at 5 % level) for an addition of one covariate into the model. Each covariate was first tested alone to assess its contribution to the model fit. Whether the magnitude of the effect was clinically meaningful was also considered before introducing the covariate into the model. Covariates were included in the order of their magnitude of impact on the PK parameter and higher drop in OFV. Once all covariates included in the model, a backward deletion of covariates was carried out. Only physiological plausible covariates associated with an increase in OFV of > 10.83 (0.1 % significant level) on their removal were retained in the final model (19).

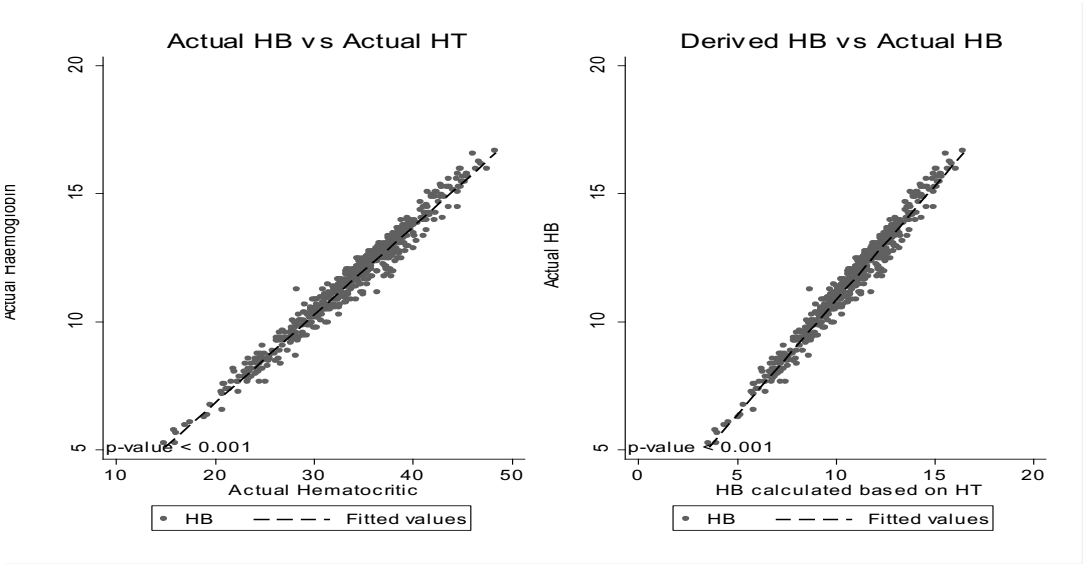

Figure 2.3 Correlation between derived HB and actual HB based on equation 3 (right panel) and correlation between actual HB and actual HT (left panel). HB is the hemoglobin in g/dL and HT is the hematocrit value in %.

Disease effect

To evaluate the disease effect (DE) on the clearance of AQ an exponential function was used (Equation 15). The fractional change (FC) of the clearance over time due to the effect of disease severity was estimated with a decay rate constant *Ke* (from which half-life of the process can be estimated from the relationship ln(2)/Ke).

$$DE = 1 - FC.exp(-K_e.t) \quad (15)$$

Hepatic extraction

A semi-physiological model with hepatic extraction (41), equation 16 was used, with the aim to describe the hepatic clearance and first-pass extraction with hepatic intrinsic clearance (CL_{int}) of AQ (42, 43). Several assumptions for this model were imposed: AQ protein binding of 90% (42) (57) which gives a fractional unbound (fu) as 10% and hepatic plasma flow (Q_H) for a typical individual of 70 kg was fixed to 100 L/h (44). The hepatic extraction ratio (E_H), the hepatic clearance (CL_H), and the fraction of absorbed drug escaping hepatic first-pass extraction (F_H) was defined according to a well-stirred model (45).

$$E_H = \frac{CL_{int} * f_u}{Q_H + CL_{int} * f_u}$$
$$CL_H = Q_H * E_H \quad (16)$$
$$F_H = 1 - E_H$$

2.3.5 Model evaluation

Both basic and advanced internal validity of the pop-PK model were used to assess the models developed. Primarily, the OFV and precision of the parameter estimates were used as basis of model diagnostics.

Basic internal validation includes the assessment of goodness-of-fit plots. These include observed (OBS) vs. individual predicted value (IPRED), OBS vs. population predicted values (PRED), individual weighted residuals (iWRES) as well as the conditional weighted residuals distribution (CWRES) vs. IPRED and observed vs predicted values. Conditional weighted residuals is more appropriate than weighted residuals, as the former is calculated based on the FOCE approximation while the latter is based on FO approximation (46).

Advanced internal validation measures include the confidence intervals (CI) estimate of the parameter using bootstrap. A non-parametric bootstrap with replacement (n = 1000) was used to evaluate the robustness of the parameter estimates of the final model. In short, non-parametric bootstrap was used to assess the precision of a population parameters without making strong distributional assumptions and using Monte Carlos simulation to repeatedly sample from the observed data with

replacement. A new set of data, namely a population of the same size as the original data, was then generated and the bootstrap estimate of the parameter of interest generated (19).

Other diagnostic tools such as visual predictive checks (47) over all datasets and stratified by study/country or age group were used to assess the accuracy and robustness of the final population model. Visual predictive check (VPC) is a model diagnostic tool which visually allows model comparison between alternative models and can be used to check if the model fits the data well. In a typical VPC (Figure 2.4) a number of stochastic simulations from the final model is performed to derive the concentration at each time point in the dataset. The distribution of the percentiles, often chosen as 5^{th}, 50^{th} (median) and 95^{th}, of the simulated data are then plotted together with the original data superimposed with 90% CI of the percentile of the simulated data (48). Data below limit of quantification can be also evaluated with the use of VPC. The 95% CI of the actual fraction of the original data are compared with the simulated fraction of BLQ data at given time periods (49).

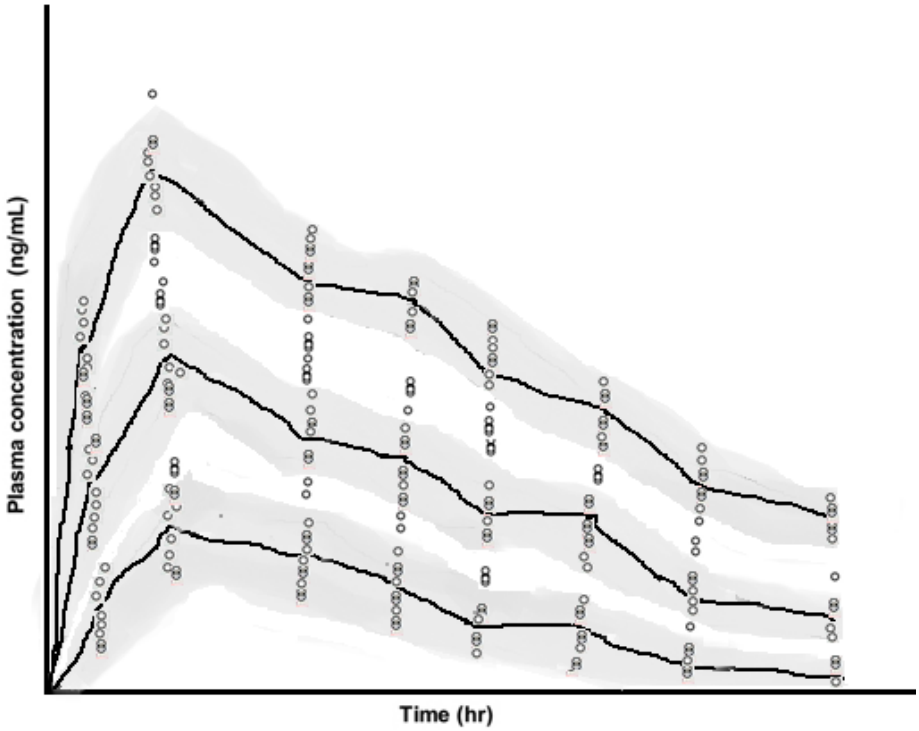

Figure 2.4 Sample of visual predictive check for pop- PK model diagnostics. The middle line is the median of the observed data, the lower and upper lines are the 5th and 95th percentiles of observed concentration. The shaded area is the 90% CI of the percentiles of the simulated data. The open circles are the individual data.

2.3.6 Simulation

Individual parameter estimates were obtained from the final PK model and used to simulate the day 7 concentration and maximum plasma concentration (C_{max}). The steady-state C_{max} was used as measure of safety. For drugs with long elimination half-life like those used in this thesis (amodiaquine and naphthoquine), the day 7 concentration could be used as the measure of exposure level representing a proxy for the efficacy of the drug. Day 7 concentrations have been shown to correlate well with the

area under the plasma concentration-time curve for naphthoquine (50). For DEAQ, a biologically active metabolite of amodiaquine (51, 52, 53), the concentration on day 7 has been reported to correlate with cure (7, 54, 55). If the C_{max} (safety) threshold was available from the manufacturers, or literature, the value could be set as the cut-off point for the safety measure during the simulation. However, a threshold value was not available, and therefore, a threshold value was obtained via simulation using a "typical subject". The maximum dose deemed safe was assigned to a representative 50 kg patient and simulated 5000 times to obtain an estimate of the median C_{max}, which was then used as a threshold value for safety. Similarly, for the efficacy threshold, the dose that resulted in a higher efficacy was used in the simulation of a typical patient (from 5000 simulations) with the median day 7 concentration estimated.

Individual demographic data from malaria patients used in this thesis came from different malaria endemic countries in Sub-Saharan African (56–59) and from routine malaria surveillance studies at the Bagamoyo research and training center (Ifakara health institute) – Tanzania (data not published). These data were used to define the characteristics of simulated patient by creating a database of *in silico* patients for simulation that cover a wide range of realistic weights and ages representing a real population and range of weights and ages recommended by the manufacturers.

In general, five thousand simulations were run using the final pop-PK models implementing the manufacturers dosing guidelines. The predicted median day 7 and C_{max} values for each weight was collated. The simulated values were compared across different weight bands as defined according to the manufacturer's recommendation and were evaluated for each available tablet strength. For each weight band a new dose was recommended if the median day 7 concentration for each weight was less than 80% of median day 7 concentration of the typical patient. While for safety concerns, the median C_{max} was required to be below the pre-defined cut-off point.

2.4 Ethics

All studies were conducted in accordance with the principles of good clinical and scientific practice and the declaration of Helsinki. The studies in chapter 3 were approved by the ethics committee of the faculty of tropical medicine, Bangkok- Thailand (MUTM 2007-112), and the Oxford tropical research ethics committee (OxTREC 024-06) in the United Kingdom (3, 4), Liberia Ministry of Health and Le comité de protection des personnes de Saint Germain en Laye (CPP), France and to the MSF ethical committee (6), the Uganda National council of science and technology; the Makerere university research and ethics committee in Uganda; and the university of California, San Francisco, committee on human research in the United States (5), the Burkina Faso ethics committee for health Research in Burkina Faso and the WHO secretariat committee on research involving human subjects

(ISRCTN07576538) (7) and the ethical and protocol review committee of the university of Ghana medical school in Ghana (2). In chapter 4 the approval was from Ghana health service ethics review committee, Navrongo Health Research Centre ethics review board and University of Cape Town human research ethics review committee and informed consent from each participant (8). While for chapter 5 the study was approved by the institutional review board of the Ifakara HealthIinstitute – Tanzania, National institute for medical research (NIMR) – Tanzania and Tanzanian food authority – Tanzania. All studies obtained the consent from the patient or the parent/guardian of the patient in case of a child.

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Chapter Three

3. Article: Population Pharmacokinetics of the Antimalarial Amodiaquine: A Pooled Analysis to Optimize Dosing

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Population Pharmacokinetics of the Antimalarial Amodiaquine: a Pooled Analysis to Optimize Dosing

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3.1 Abstract

Amodiaquine plus artesunate is the recommended antimalarial treatment in many countries where malaria is endemic. However, pediatric doses are largely based on a linear extrapolation from adult doses. We pooled data from previously published studies on the pharmacokinetics of amodiaquine, to optimize the dose across all age groups. Adults and children with uncomplicated malaria received daily weight-based doses of amodiaquine or artesunate-amodiaquine over 3 days. Plasma concentration-time profiles for both the parent drug and the metabolite were characterized using nonlinear mixed-effects modeling. Amodiaquine pharmacokinetics were adequately described by a two-compartment disposition model, with first-order elimination leading to the formation of desethylamodiaquine, which was best described by a three-compartment disposition model. Body size and age were the main covariates affecting amodiaquine clearance. After adjusting for the effect of weight, clearance rates for amodiaquine and desethylamodiaquine reached 50% of adult maturation at 2.8 months (95% confidence interval [CI], 1.5 to 3.7 months) and 3.9 months (95% CI, 2.6 to 5.3 months) after birth, assuming that the baby was born at term. Bioavailability was 22.4% (95% CI, 15.6 to 31.9%) lower at the start of treatment than during convalescence, which suggests a malaria disease effect. Neither the drug formulation nor the hemoglobin concentration had an effect on any pharmacokinetic parameters. Results from simulations showed that current manufacturer dosing recommendations resulted in low desethylamodiaquine exposure in patients weighing 8 kg, 15 to 17 kg, 33 to 35 kg, and >62 kg compared to that in a typical 50-kg patient. We propose possible optimized dosing regimens to achieve similar drug exposures among all age groups, which require further validation.

KEYWORDS NONMEM, dose optimization, malaria, pediatrics

3.2 Introduction

Approximately 3.2 billion people were at risk of *Plasmodium falciparum* malaria in 2015, with an estimated 212 million malaria cases and 429,000 malaria-related deaths occurring that year. Children under 5 years of age carry the highest burden of disease, accounting for 70% of malaria-related deaths in 2015 (1). Artemisinin resistance has been confirmed in at least six countries in Southeast Asia (2,–4). Thus, both the choice of and correct dosing of artemisinin-based combination therapies (ACTs) are crucial and should be driven by a comprehensive understanding of the pharmacokinetic (PK) and pharmacodynamic (PD) properties of both drugs in the combination.

Artesunate plus amodiaquine is one of the five ACTs currently recommended by the World Health Organization (WHO) and has been adopted as a first-line or second-line treatment in at least 25 countries (5). Four different amodiaquine-based treatments are available, including amodiaquine alone, coblistered amodiaquine plus artesunate (loose combination), an amodiaquine-plus-artesunate fixed-dose combination, and coblistered amodiaquine plus sulfadoxine-pyrimethamine. A recent meta-analysis showed that amodiaquine plus artesunate administered in a fixed-dose formulation results in efficacy higher than that of the other formulations (6).

Amodiaquine is primarily metabolized in the liver by cytochrome P450 (CYP) 2C8 (CYP2C8) to its biologically active metabolite, desethylamodiaquine (7,–9), which is thought to be the driver of the antimalarial activity (8, 10). Desethylamodiaquine is eliminated slowly and has been detected in plasma and blood for up to 1 month after drug administration (10). Desethylamodiaquine is further metabolized *in vivo*, via an unknown route, into its inactive metabolite, bis-desethylamodiaquine (11). Both amodiaquine and desethylamodiaquine are over 90% protein bound (12) to α1-acid glycoprotein (13).

Several studies have investigated dosing regimens for current antimalarial combination therapies (14,–19). There is some concern that dosing recommendations may not be optimal for some subgroups of patients, such as young and/or malnourished children and pregnant women, due to

altered PK properties in these vulnerable groups. Several studies have investigated the PK of amodiaquine and desethylamodiaquine using both model-based and model-independent analyses (20,–25). However, to the best of our knowledge, no studies have investigated the population PK of amodiaquine and desethylamodiaquine across a broad range of ages by combining individual patient-level data from different studies. Pooling of data from different populations allows for the assessment of clinically important determinants affecting PK parameters that might not be possible to investigate in smaller individual studies, and the large amount of data provides more accurate and reliable parameter estimates.

The objective of this analysis was to investigate the PK of amodiaquine and desethylamodiaquine by pooling data from previously published studies, using nonlinear mixed-effects modeling. The developed model was used to assess the current dosing recommendations and to propose an improved dosing regimen, if necessary.

(This analysis was part of Ali Mohamed Ali's Ph.D. work, so the information in this article will be included in his Ph.D. dissertation.).

3.3 Results

Thirteen relevant clinical studies were identified, of which eight were shared with the WorldWide Antimalarial Resistance Network (WWARN). Two studies were contributed after the database for analysis was locked and were therefore not included. Data from 261 patients were available for PK analysis. Of these patients, 95 (36.4%) were children under 5 years of age and 26 were pregnant women (21, 24). The median age at the baseline across the studies was 7.6 years (interquartile range [IQR], 3.6 to 18 years; range, 1 to 60 years). All patients were infected with Plasmodium falciparum, except for those in one study cohort from Asia (21, 24), which had Plasmodium vivax monoinfections. The geometric mean baseline parasitemia was 24,400 parasites/µl of blood (IQR, 7,880 to 76.500 parasites/µl of blood; range, 240 to 566,000 parasites/µl of blood) and 114 parasites/µl of blood (IQR, 288 to 3,920 parasites/µl of blood; range, 96 to 50,500 parasites/µl of blood) in patients infected with Plasmodium falciparum and Plasmodium vivax, respectively. Baseline values for each study population are presented in Table 1, and detailed information can be found in the individually published reports (20,–25). Children from Burkina Faso (who were among the youngest) received a higher total dosage (median, 33.8 mg/kg of body weight; range, 14.8 to 65.6 mg/kg) than the other study populations (Kruskal-Wallis test, $P < 0.001$) (Table 1).

Table 3.1 Patient characteristics at baseline[a]

Characteristic	Value(s) for the following study(ies):					Total
	Tarning and colleagues (96, 97)	Jullien et al. (99)	Mwesigwa et al. (98)	Stepniewska et al. (100)	Adjei et al. (95)	
No. of patients	26 (+7)[b]	53	20	61	101	261
% of male patients (no. of male patients/total no. of patients)	0 (0/26)	47.2 (25/53)	65.0 (13/20)	54.1 (33/61)	50.5 (51/101)	46.7 (122/261)
Median (range) age (yr)	23.0 (16.0 – 39.0)	24.0 (18.0 – 60.0)	9.0 (6.0 – 13.0)	2.5 (1.0 – 5.0)	6.0 (1.0 – 14.0)	7.6 (1.0 – 60.0)
% of patients aged (yr) (no. of patients of the indicated age/total no. of patients):						
< 2	0 (0/26)	0 (0/53)	0 (0/20)	32.8 (20/61)	12.9 (13/101)	12.6 (33/261)
2 to < 5	0 (0/26)	0 (0/53)	0 (0/20)	65.6 (40/61)	21.8 (22/101)	23.8 (62/261)
5 to < 12	0 (0/26)	0 (0/53)	90.0 (18/20)	1.6 (1/61)	53.5 (54/101)	28.0 (73/261)
12+	100 (26/26)	100 (53/53)	10.0 (2/20)	0 (0/61)	11.9 (12/101)	34.6 (93/261)
% of patients receiving the following drug formulation, treatment regimen (no. of patients receiving the formulation/total no.):						
AQ+AS, FDC	0 (0/26)	47 (25/53)	0 (0/20)	47.5 (29/61)	0 (0/101)	20.7 (54/261)
AQ + AS, Separate tablets	0 (0/26)	53 (28/53)	100 (20/20)	52.5 (32/61)	85.2 (86/101)	63.6 (166/261)
AQ alone	100 (26/26)	0 (0/53)	0 (0/20)	0 (0/61)	14.8 (15/101)	15.7 (41/261)
Enrolment demographic, vital, and laboratory parameters						
Median (range) wt (kg)	49.0 (37.0 – 68.0)	59.0 (39.0 – 90.0)	24.5 (20.0 – 42.0)	12.5 (7.0 – 31.0)	18.0 (6.5 – 93.0)	21.0 (6.5 – 93.0)
Median (range) total dose (mg/kg)	30.5 (28.1 – 63.0)	29.5 (18.0 – 47.1)	24.3 (23.9 - 26.0)	33.7 (14.8 – 65.6)	30.0 (30.0 - 30.0)	30.0 (14.8 – 65.6)
Geometric mean (range) parasitemia (no. of parasites/µL)	1,142 (96 – 50, 453)[c]	11,923 (1,127 – 10,9356)	11,122 (240 – 174,800)	23,110 (1,357 – 467,600)	42,689 (630 – 566,358)	17,952 (96 – 566,358)

Median (range) hematocrit (%)	32.5 (23.0 – 40.0)	-	-	-	-	32.5 (23.0 – 40.0)
Median (range) hemoglobin concn (g/dL)	10.3 (6.7 – 13.2)[d]	13.2 (9.9 – 17.7)	12.2 (9.7 – 14.1)	8.7 (5.9 – 12.4)	11.6 (6.5 – 15.1)	11.2 (5.9 – 17.7)

[a]Percentages can be more than 100% due to rounding errors. AQ, amodiaquine; AS, artesunate; FDC, fixed-dose combination.

[b]Seven patients were sampled again after delivery, during another episode of malaria.

[c]The data are for patients with vivax malaria.

[d]Derived on the basis of their hematocrit value.

A total of 2,920 postdose venous plasma samples for both amodiaquine and desethylamodiaquine were collected, but the majority of patients (162/261; 62.1%) contributed only 1 to 3 samples. Three studies (147 patients) collected samples for drug measurements before the first study dose, but only 1 (0.7%) amodiaquine concentration and 29 (19.7%) desethylamodiaquine concentrations were measured to be above the lower limit of quantification (LLOQ) (Table 2). Four (0.3%) samples for amodiaquine and 37 (2.5%) samples for desethylamodiaquine were excluded from the analysis because of unreliable information on the time of sample collection and/or because the findings for those samples were deemed biologically implausible after inspection of the PK profile. In the final analysis, 1,456 (99.7%) samples for amodiaquine and 1,423 (97.5%) samples for desethylamodiaquine were included. Out of these, the concentrations in 803 (55.2%) samples for amodiaquine and 13 (0.9%) samples for desethylamodiaquine were below the LLOQ.

Population pharmacokinetic model. The observed amodiaquine data were best described by a two-compartment disposition model, and two transit compartments successfully described the absorption phase, while desethylamodiaquine was best described by three-compartment disposition kinetics. Replacement of the structural model with a semimechanistic hepatic model did not result in a better fit and was not pursued further. The use of the M3 method to handle LLOQ values did not significantly affect the population parameter estimates, but the model estimation process was unstable and required much longer run times than the M6 method, which was therefore used throughout the modeling process.

Table 3.2 Descriptions of the population pharmacokinetic and noncompartmental studies[a]

Country	Study description (authors [reference(s)])	Treatment (protocol)	Study population	Formulation	Manufacturer	No. of patients	Sampling schedule (Protocol)	Sample collection	Sample storing and assay	No. of samples per patient[b]	LLOQ - AQ/DEAQ concn (ng/ml)
Thailand[c]	Effect of pregnancy on PK and PD of Amodiaquine and Desethyl amodiaquine (Tarning and colleagues [20, 23])	AQ (10 mg/kg) daily for 3 days, 200 mg amodiaquine hydrochloride (153 mg amodiaquine base)	Pregnant women (ages, 16 to 39 yr) in their 2nd and 3rd trimester (with follow-up after delivery)	AQ alone	Sanofi-Aventis, France	26 (7)[d]	0, 4, 24, 28, 48, 48.5, 49, 50, 51, 52, 54, 56, 58, and 72 h; 4, 5, 7, 14, 21, 28, 35, and 42 days	A sample was drawn from a catheter during the first 3 days and thereafter by venous puncture and placed into lithium heparin tubes	Samples were stored at -20°C and analyzed by LC-MS/MS	14/22 (14/22)	1/2
Kenya[c]	Efficacy of fixed vs nonfixed dose of ASAQ (Jullien et al. [22])	Two tablets of AS-AQ at a fixed dose (100/270 mg) or 4 tablets of AS (50 mg) + 4 tablets of AQ (153 mg) daily for 3 days, 353/200 mg amodiaquine hydrochloride (153/270 mg amodiaquine base)	Adults (ages, 18 to 60 yr)	AS + AQ at a fixed dose and as a loose formulation	Sanofi-Aventis, France	53	Before 1st dose, 15 min to 4 h after 1st dose, 15 min to 4 h after 2nd dose, just before 3rd dose, 15 min to 4 h after 3rd dose, days 7, 14, 21, and 28		Samples were analyzed by HPLC	4/8	1/1
Uganda[e]	Determine PK parameters for AS and AQ in children (Mwesigwa et al. [24])	AS (50-mg tablets at 4 mg/kg twice a day for 3 days) + AQ (200-mg tablets at 10 mg/kg once a day on the first 2 days and 5 mg/kg on the third day), 200 mg amodiaquine hydrochloride (153 mg amodiaquine base)	Children (ages, 5 to 13 yr)	AS + AQ, loose formulation	Sanofi-Aventis, France	20	Just prior to 3rd dose and at 2, 4, 8, 24, and 120 h after 3rd dose	A venous sample was drawn into potassium oxalate-sodium fluoride tubes	Samples were stored at -80°C and analyzed by LC-MS/MS	2/6	5/5
Burkina Faso[c]	Compare bioavailability of fixed doses of AS and AQ vs AS and AQ separately (Stepniewska et al. [21])	AS-AQ at a fixed dose (one dose of 25/67.5 mg/kg for children <12 mo of age or two doses for children ages 12 to 60 mo) or AS (50-mg tablet, a half tablet for children <12 mo of age and one tablet for children ages 12 to 60 mo) + AQ (153 mg, a half tablet for children <12 mo of age and one tablet for children ages 12 to 60 mo) daily for 3 days, 200 mg amodiaquine hydrochloride (153 mg amodiaquine base)	Children (ages, 1 to 5 yr)	AS + AQ at a fixed dose and as a loose formulation	Sanofi-Aventis, France	61	Before the 1st dose, 4 h after the 3rd dose, and then at days 7 and 14 and days 21 and 28	A venous sample was collected in lithium heparin tubes	Samples were stored at -20°C and analyzed by LC-MS/MS	2/3	1/1

Ghana[c]	Compare the effect of AS on AQ (Comparison between AQ and loose formulations of AS and AQ) (Adjei et al. [19])	AQ (10 mg/kg single dose) or AS (4-mg/kg single dose) + AQ (10-mg/kg single dose) daily for 3 days, 200 mg amodiaquine hydrochloride (153 mg amodiaquine base)	Children (ages, 1 to 14 yr)	AS + AQ, loose formulation	Pfizer, Dakar, Senegal	101	Before the dose on days 3 and 7	A venous sample was collected into heparinized polypropylene tubes	Samples were stored at -20°C and analyzed by HPLC	1/2	10/10

[a]All samples were venous plasma. AS, artesunate; AQ, amodiaquine; LLOQ, lower limit of quantification; DEAQ, desethylamodiaquine; LC-MS/MS, liquid chromatography-tandem mass spectrometry; HPLC, reverse-phase high-performance liquid chromatography.

[b]Median number of Amodiaquine/Des-ethyl amodiaquine samples per subject; values in parentheses are the same women at 3 moths postdelivery.

[c]Population PK study.

[d]The same women were sampled again at 3 months postdelivery during another malaria episode.

[e]Noncompartmental pharmacokinetic analysis.

The final structural model is depicted in Fig. 1, and the parameter estimates, together with their precision obtained from a nonparametric bootstrap, are reported in Table 3.

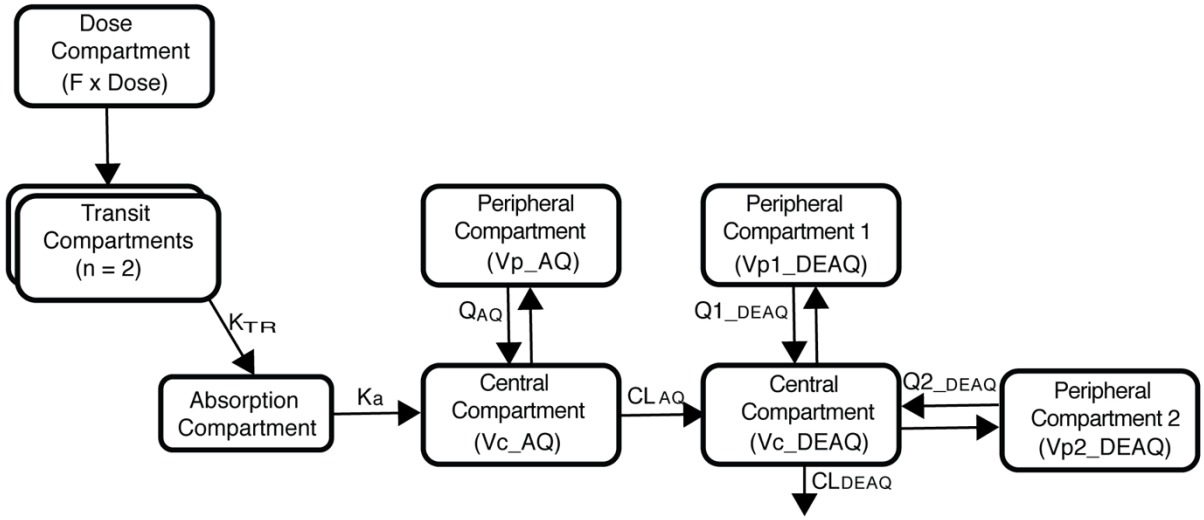

Figure 3.1 Structure of the PK model of amodiaquine and desethylamodiaquine. Abbreviations: *F*, oral bioavailability; K_{TR}, first-order transit rate constant; *Ka*, absorption rate constant; AQ, amodiaquine; DEAQ, desethylamodiaquine; CL, clearance; *Vc*, central volume of distribution; *Q*, *Q1*, *Q2*, inter-compartmental clearances; *Vp*, *Vp1*, *Vp2*, peripheral volumes of distribution.

Table 3.3 Parameter estimates of the population pharmacokinetic model for amodiaquine and desethylamodiaquine

Parameter	Typical value[a]		BSV or BOV[a,b]				
	Value (% RSE)	95% CI	BSV (% RSE)	BOV (% RSE)	% shrinkage		95 % CI
					Eta	Epsilon	
Amodiaquine							
K_a (1/h)	0.589 (23)	0.409, 0.905		78.5 (12)	41.7		62.0, 96.5
MTT (h)	0.236 (26)	0.161, 0.334		93.4 (16)	61.8		60.6, 120
NN	2.00 (78)	1.09, 6.31					
F	1 Fixed			30.9 (8.0)	27.1		26.5, 36.0
CL_{AQ}[c] (liters/h)	2,960 (4.4)	2,600, 3,118	32.2 (13)		44.3		24.2, 39.5
Vc_{AQ}[c] (liters)	13,500 (19)	7,423, 17,824	53.1 (30)		55.4		19.4, 79.5
Q_{AQ}[c] (liters/h)	2,310 (9.2)	1,877, 2,722					
Vp_{AQ}[c] (liters)	22,700 (10)	17,956, 27,114					
Additive error[d] (ng/mL)	LLOQ/5 + 0.445 (19)	LLOQ/5 + 0.249, 0.609				26.7	
Proportional error (%)	19.9 (11)	16.1, 24.6					
Desethylamodiaquine							
CL_{DEAQ}[c] (liters/h)	32.6 (2.9)	29.7, 33.4	20.0 (10)		31.3		15.5, 23.5
Vc_{DEAQ}[c] (liters)	258 (12)	201, 318	67.2 (21)		59.3		36.0, 89.2
Q_{1DEAQ}[c] (liters/h)	154 (6.6)	131, 171					
Vp_{1DEAQ}[c] (liters)	2,460 (5.9)	2,129, 2,677					
Q_{2DEAQ}[c] (liters/h)	31.3 (6.2)	26.8, 34.3					
Vp_{2DEAQ}[c] (liters)	5,580 (4.3)	4,968, 5,904					
Additive error[d] (ng/mL)	LLOQ/5 Fixed					16.5	
Proportional error (%)	24.2 (4.0)	22.2, 25.9					

Covariate effects		
PMA_{50} for AQ[e] (time [mo] from conception)	11.8 (4.6)	10.50, 12.70
Hill factor for AQ[e]	3.6 (4.0)	3.23, 3.80
PMA_{50} for DEAQ[e] (time [mo] from conception)	12.9 (5.7)	11.60, 14.30
Hill factor for DEAQ[e]	3.22 (4.7)	2.85, 3.43
Effect of first dose on F (%)	-22.4 (19)	-32.0, -15.6

[a]The precision of the parameter estimates was assessed using a nonparametric bootstrap of the final model (n = 500). The relative standard errors were calculated as 100 × (standard deviation/mean), while the confidence intervals were obtained on the basis of the empirical percentiles of the bootstrap estimates.

[b]Between-subject and between-occasion variability were assumed to be log-normally distributed and are reported as the approximate percent coefficient of variation.

[c]All clearances and volumes of distribution refer to a patient weighing 50 kg, the median weight in the data set.

[d]For the data contributed by each study, the additive error was fixed to 20% of the lower limit of quantification (LLOQ) in that study plus an estimated parameter. For desethylamodiaquine, this extra parameter was not significantly different from zero, so the additive error was fixed to the lower bound of LLOQ/5.

[e]Estimated using prior functionality in NONMEM.

[f]Abbreviations: AQ, amodiaquine; DEAQ, desethylamodiaquine; BSV, between-subject variability; BOV, between-occasion variability; RSE, relative standard error; K_a, absorption rate constant; MTT, mean transit time; NN, number of transit compartments; F, relative bioavailability; CL, clearance; Vc, central volume of distribution; Q, Q_1, and Q_2, intercompartmental clearances; V_p, V_{p1}, and V_{p2}, peripheral volumes of distribution; PMA_{50}, time to reach 50% of clearance maturation; Hill factor, steepness of the clearance maturation curve.

The model adequately described all observed data from the included studies, as shown by the study-stratified visual predictive check (VPC) plots in Fig. 2. VPCs stratified by age and basic goodness-of-fit diagnostic plots are presented in Fig. S1 and S2 in the supplemental material, respectively. Even though some small trends can be observed for amodiaquine for the first hours after the dose in some of the contributed studies, these plots showed no overall obvious model misspecification and suggested that the developed model has adequate predictive performance, especially for desethylamodiaquine, which is the main driver of the therapeutic outcome.

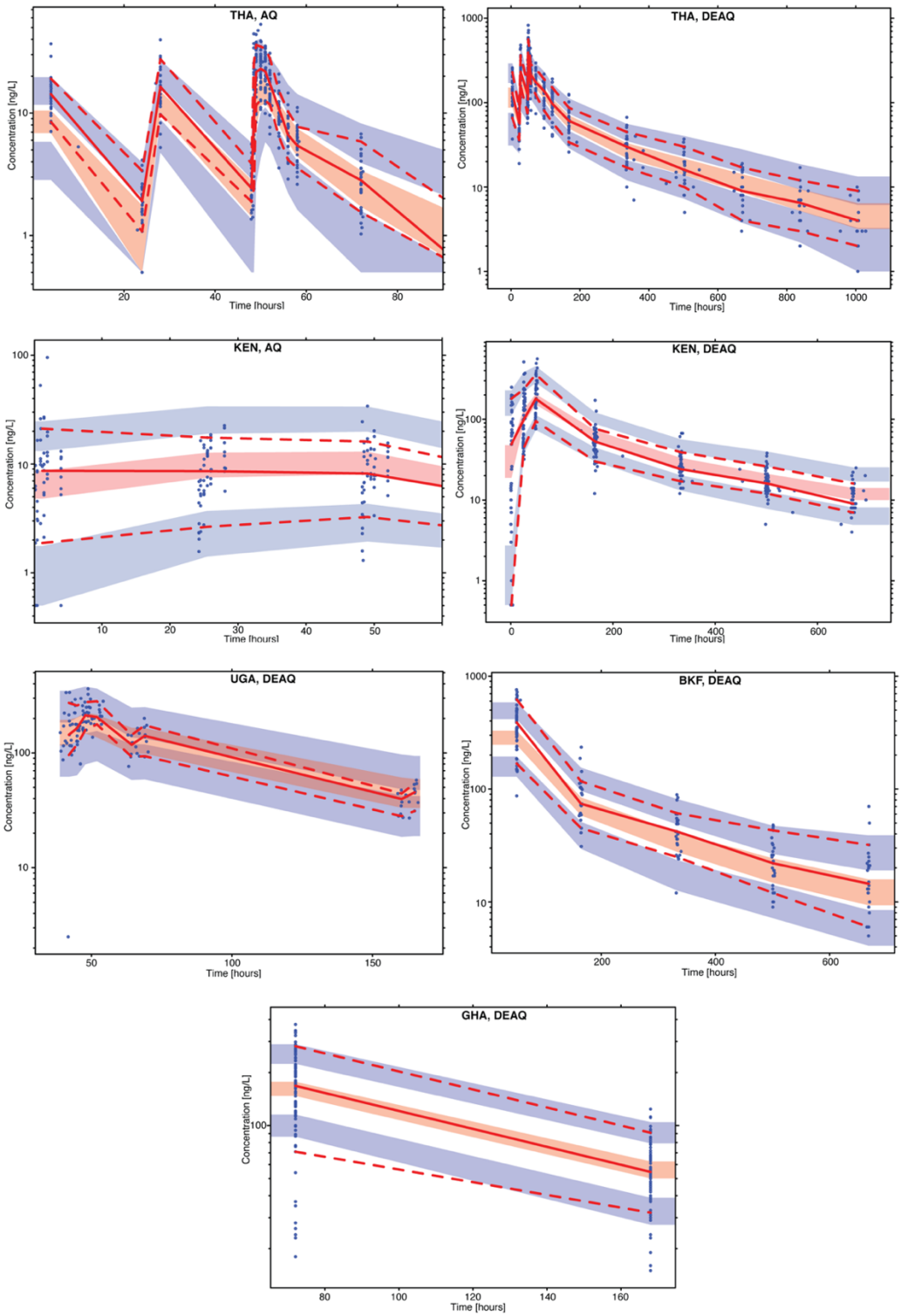

Figure 3.2 Visual predictive check of the final model describing the plasma concentrations of amodiaquine (AQ) and desethylamodiaquine (DEAQ) versus time in uncomplicated malaria patients from Thailand (THA), Kenya (KEN), Uganda (UGA), Burkina Faso (BKF), and Ghana (GHA). Open circles are the observed data points; solid and dashed lines are the 50th, 5th, and 95th percentiles of the observed data; shaded areas are the simulated (n=1000) 95% confidence interval for the same percentile. The *y* axis represents the plasma concentration on the log scale. Censored data points below the lower limit of quantification were imputed as LLOQ/2 and included in the calculation of percentiles for the observed and the simulation data. The VPC for amodiaquine for both Thailand and Kenya was cut at time 90 and 60 h, respectively, since beyond these times the concentrations from both observed and simulated data were below the LLOQ.

Covariate effect. Allometric scaling with body weight on all clearance and volume parameters improved the model fit substantially (change in the objective function value [ΔOFV] = −446). Inclusion of a maturation function for the clearance of desethylamodiaquine improved the model further (ΔOFV = −34.6; 2 degrees of freedom [df]; P < 0.001) and decreased the between-subject variability (BSV) of desethylamodiaquine clearance from 24% to 19.1%. A similar maturation effect did not reach statistical significance for amodiaquine (ΔOFV = −3.1; 2 df; P = 0.21) and decreased the BSV on clearance only slightly, from 62.6% to 61.7%, but it was retained in the final model for consistency between the parent compound and the metabolite. The final parameter estimates of the maturation function were close to the prior values used to stabilize the model; clearance was found to reach 50% of weight-adjusted adult values at 2.8 months of age (95% confidence interval [CI], 1.5 to 3.7 months of age) and 3.9 months of age (95% CI, 2.6 to 5.3 months of age) (assuming a standard 9-month gestation) for amodiaquine and desethylamodiaquine, respectively. The maturation effect is displayed in Fig. 3, which shows that 95% of the adult value is reached by 2 years of age. Finally, the model estimated the bioavailability of the first dose in the treatment regimen to be 22.4% (95% CI, 15.6 to 31.9%) lower than that on the second and third days of treatment (ΔOFV = −18.9; 1 degree of freedom; P < 0.001; decrease in the between-occasion variability [BOV] on bioavailability, 33% to 31%). Inclusion of the drug formulation and hemoglobin (HB) concentration on bioavailability did not improve the model fit, nor did the inclusion of the hemoglobin concentration on amodiaquine or desethylamodiaquine clearance.

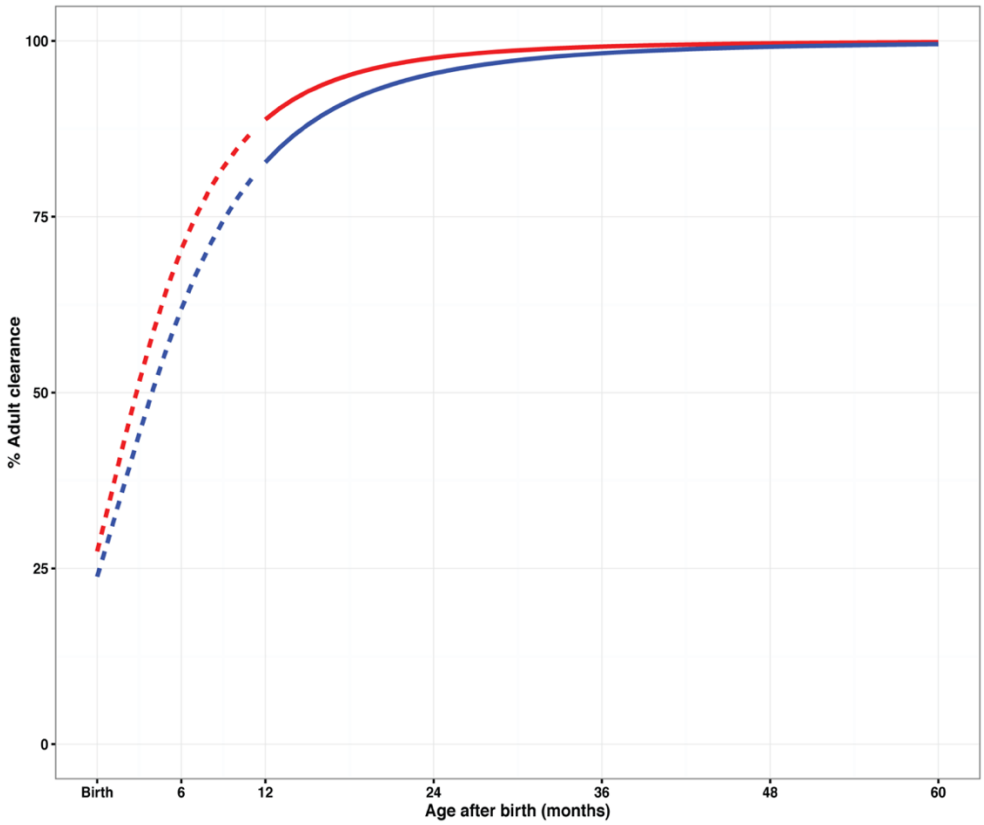

Figure 3.3 Clearance maturation for amodiaquine (red line) and desethylamodiaquine (blue line) expressed as a fraction of adult clearance predicted from PK model plotted against post-natal age (assuming birth occurred at term). The dashed lines indicate the section of the maturation curve that falls in the age range below that observed in study data and therefore based on an extrapolation.

Variability. Even after adjusting for all covariate effects described above, a large amount of BSV and BOV was still identified. A large BOV in absorption parameters (>78%), a large BSV in the central volume of distribution for both amodiaquine and desethylamodiaquine (>50%), a moderate BSV in clearance for both amodiaquine and desethylamodiaquine (20 to 33%), and a moderate BOV for bioavailability (30.9%) were observed. Moderate to high eta shrinkages (30 to 60%) were observed for parameter estimates, while epsilon shrinkages were low (<30%), as summarized in Table 3.

Simulations. The final model was used to evaluate the exposure achieved with the current dosing recommendations (26), and the results are summarized in Fig. 4A. Children who weigh 8 kg, 15 to 17 kg, or 33 to 35 kg and patients who weigh >62 kg achieve desethylamodiaquine day 7 concentrations that are, on average, 25% lower than the concentration for a 50-kg patient. Population-based simulations using the final PK model suggested that higher dosages are needed to achieve equivalent exposures in all body weight groups, raising the dosing recommendations for amodiaquine from 7.5 to 16.9 mg/kg/day to 9.8 to 19.9 mg/kg/day in certain patient groups (Fig. 4; Table 4). It is important to note that Fig. 4 shows simulated patients, including some with ages below the age range of the observed data.

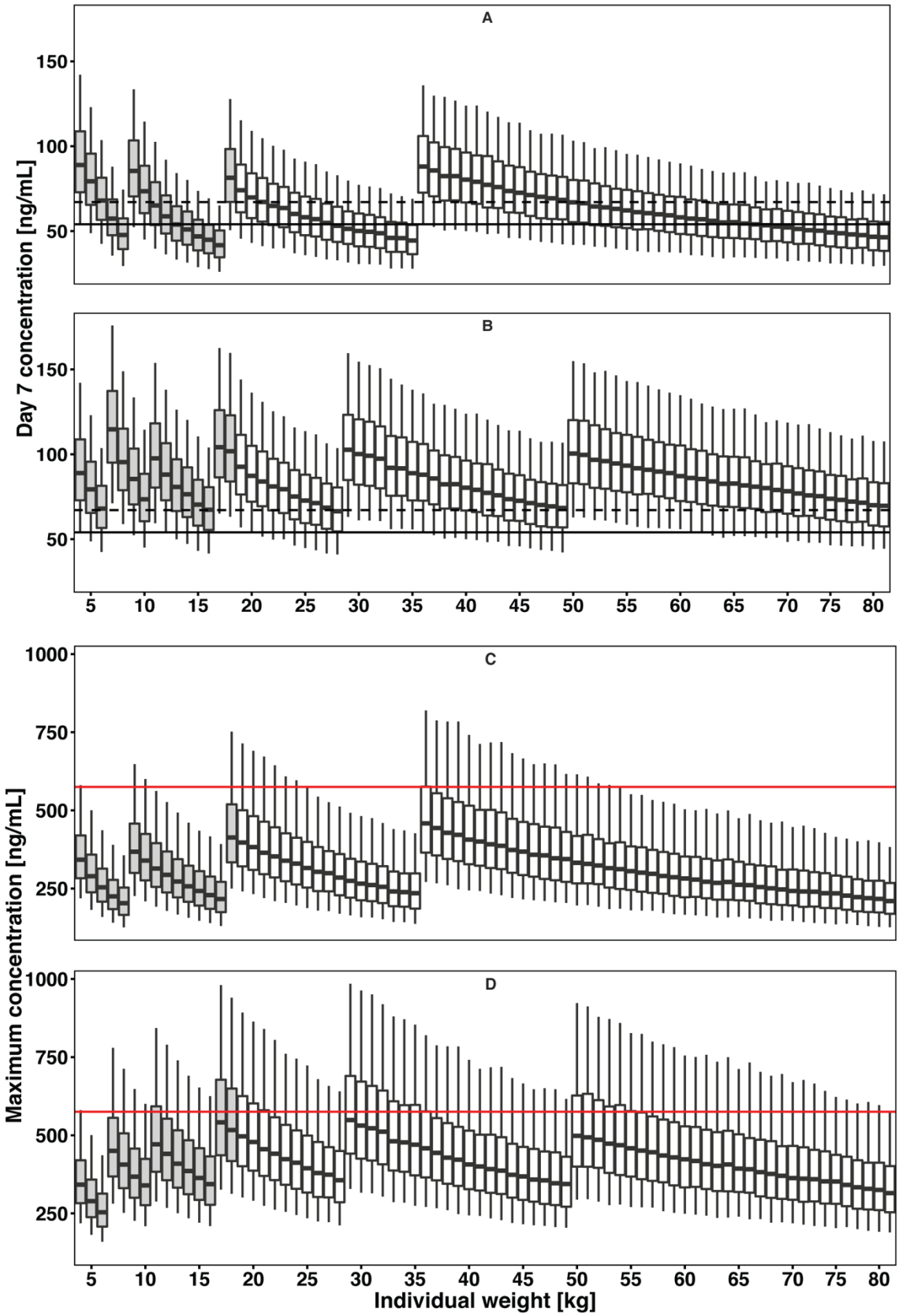

Figure 3.4 Simulation results of current recommended and optimized dose regimens for amodiaquine. (A and C) Day 7 plasma desethylamodiaquine concentration (A) and maximum concentration of desethylamodiaquine (C) based on the current recommended dose regimen. (B and D) Predicted desethylamodiaquine day 7 concentration for the optimized dose regimen

designed to achieve a concentration ≥75% above the threshold value (B) and C_{max} of desethylamodiaquine for the optimized dose regimen (D). The black dashed and solid lines in panels A and B are the median and 80% value of median of the simulated day 7 plasma desethylamodiaquine concentration for the typical patient (representing the expected exposure level from the current dosing recommendation), respectively, and the red lines in panels C and D represents the C_{max} upper threshold (575 ng/ml). Simulations for each weight are presented as a box plot for the median and 25th and 75th percentiles, with whiskers representing the 5th and 95th percentiles. The boxes in gray indicate that the simulation for that age range is based on an extrapolation, since no PK data for children of that age were available.

Table 3.4 Current dose regimen and optimized dose regimen based on simulations for amodiaquine

Current manufacturer dose regimen			Simulated based dose regimen		
Body weight (Kg)	Number of tablets/day	AQ mg	Body weight (Kg)	Number of tablets/day	AQ mg
≥ 4.5 to < 9	1 x 67.5	67.5	≥ 4.5 to < 7	1 x 67.5	67.5
≥ 9 to < 18	1 x 135	135	≥ 7 to < 12	1 x 135	135
≥ 18 to < 36	1 x 270	270	≥ 12 to < 16	1 x 135 + 1 x 67.5	202.5
≥ 36	2 x 270	540	≥ 16 to < 26	1 x 270	270
			≥ 26 to < 41	1 x 270 + 1 x 135	405
			≥ 41 to < 63	2 x 270	540
			≥ 63	3 x 270	810

[a]A substantial number of simulated patients (78%) in this weight band were younger (< 1 year) than those available in the dataset.

[b]A proportion of the simulated patients (38%) in this weight band were younger (< 1 year) than those available in the dataset.

[c]In these weight bands, as an alternative to spilling tablets, one could suggest using tablets of different strength. Either option is viable, according to the preference of the caregiver.

The predicted lower target concentration for efficacy, i.e., 80% of the median desethylamodiaquine concentration at day 7 in a 50-kg patient taking 540 mg daily for 3 days (15 mg/kg/day), was 54.0 ng/ml. The proposed optimized dosing regimen (Table 4) resulted in equivalent exposures across weight bands (Fig. 4B). In particular, higher milligram-per-kilogram doses were needed for small children, consistent with the nonlinear effect of body size on clearance, described by allometric scaling.

3.4 Discussion

This is the first pooled population PK analysis of amodiaquine and desethylamodiaquine. The analysis used individual patient data from six studies (five cohorts) of the antimalarial therapy amodiaquine, given alone or in combination with artesunate (20,–25), covering patient populations with a large range of characteristics (in terms of weight, age, and ethnicity) and covering a large range of treatments and study protocols. Consistent with other studies, the final PK model included a two-compartment model for the amodiaquine concentration-time profile and a three-compartment model for desethylamodiaquine. In addition to including the effect of body size on the clearance of both compounds with allometric scaling, the maturation profile of the clearance of both compounds was included in the model to describe the kinetics in children. To ensure exposure equivalent to that in adults, and hence assuming that this would lead to improved efficacy and curative concentrations, simulations with the final model of desethylamodiaquine indicated that higher doses of amodiaquine may be needed for some weight ranges (8 kg, 15 to 17 kg, 33 to 35 kg, and >62 kg), but this finding needs further validation.

The effect of the drug formulation or coadministration with artesunate on bioavailability was not significant in this analysis. Previously, efficacy was found to be higher for amodiaquine when given as a fixed-dose formulation with artesunate (6), suggesting that a fixed-dose formulation may directly increase bioavailability or improve adherence, leading to higher exposure, as measured by the area under the plasma concentration-time curve (AUC). Only a few patients included in the analysis received amodiaquine alone; this group included all of the patients from the Thailand study and only 6% (15/256) of the patients from studies conducted in Africa. Moreover, the patients treated with amodiaquine alone received a higher dose on a milligram-per-kilogram basis than those that received the loose formulations or the fixed-dose combination therapy. These two factors possibly limited the power of the analysis to explore formulation effects. Similarly, all pregnant patients and those infected with *Plasmodium vivax* were from one study in Asia; thus, there was little power to separate study-specific effects from any pregnancy, regional, or *Plasmodium* species effect on the PK parameters.

The structural PK model identified here matches that proposed by Tarning et al. (21) in pregnant women in Thailand. This is not surprising, since their study contributed the most intensively sampled PK profiles included in this pooled analysis. Parameter estimates were similar between the two analyses, but the central volume of distribution was larger in this pooled analysis, and, as expected, pooling of data from several studies resulted in a larger BSV (21). This could be due to differences in the body size and composition between the Asian and African populations, or it could simply be a consequence of different study sample sizes. Unfortunately, patient height data were unavailable from the African studies, and other body size descriptors (i.e., fat-free mass or body mass index) could not be explored as an alternative to total body weight.

Amodiaquine bioavailability was found to be 22.4% lower on the first day of treatment than on the second and third days, possibly due to an increased absorption of amodiaquine as a result of treatment and a general improvement in malaria disease status. Winstanley et al. (27) reported lower AUCs for amodiaquine in malaria patients than in healthy volunteers, and this may be partly explained by the lower bioavailability due to a disease effect that is more pronounced on the first day than during convalescence. Similarly, a nearly 2-fold (1.72-fold) relative increase in bioavailability has been observed in studies of mefloquine, where administration is delayed until 48 hours after the first dose of artesunate (28). A similar increase in oral bioavailability during treatment has been demonstrated in malaria patients receiving dihydroartemisinin-piperaquine and for naphthoquine when given in combination with artemisinin in pediatric malaria patients (29,–32). In contrast, a higher bioavailability was estimated for artesunate during the acute malaria phase than during the convalescent phase in a recent study (33). Taken together, there might be an emerging trend of lower bioavailability during acute phases of malaria illness for the longer-acting compound in ACT treatments, warranting further investigation. A similar parallel has been noted with antibiotic use in patients with sepsis (34).

The disease state may also alter absorption parameters (35), but the current analysis could not assess the effect of acute illnesses on absorption, as it included only malaria patients and not healthy

individuals. There was considerable heterogeneity in malaria symptoms and parasitemia, but attempts to include an effect of various parasite densities in the model were unsuccessful, possibly because it was confounded by other factors. The principal plasma protein binding site for amodiaquine is in α_1-acid glycoprotein (13), which is upregulated during malaria (36, 37), resulting in increased binding of the drug to this protein. The variation in α_1-acid glycoprotein levels could also explain part of the high between-subject variability in the apparent volume of distribution found in our analysis.

The large estimates of between-occasion variability could be a result of sparse data, and some of the studies (23, 25) included in this analysis did not measure drug concentrations on the first and second days of treatment and/or provided only approximate information about the timing of sample collection (i.e., only the day and not the exact time was available). Despite this, the effect of lower oral bioavailability for the first dose was confirmed even in a separate analysis executed only on data from studies that included sufficient information.

PK parameter estimates from adults, adjusted for the effects of body weight and size by allometric scaling, often provide poor predictions of drug clearance in young children (38), especially for those less than 1 year of age. This is because PK can change rapidly with postmenstrual age (PMA) (39,–41) in the first years of life. Age maturation of clearance rates has been reported for various pediatric drugs (16, 42,–45). In addition, the clearance of amodiaquine depends on the enzyme CYP2C8, which is closely related to CYP2C9 (46), whose isozymes are known to exhibit maturation effects (38). Similar to the approach used in previous studies of other drugs (42, 44), a sigmoidal maximum-effect function was used to model the maturation of clearance as a function of PMA. No previous estimates have been presented for amodiaquine or desethylamodiaquine, but the estimates of the PMA at which 50% enzyme maturation is achieved (PMA_{50}) presented here result in half-maximum maturation of amodiaquine and desethylamodiaquine clearance at 2.8 and 3.9 months after birth, respectively, for babies born at term. This is in line with the range reported for other drugs (0.7 to 12 months after birth, respectively, for babies born at term), as summarized by Holford et al. (47). The estimates of the

Hill coefficients were also consistent with those reported in previous studies (2 to 4), indicating a relatively sharp maturation curve plateauing at about 2 years after birth. Amodiaquine and desethylamodiaquine clearance reached at least 95% of the adult values at approximately 21 months after birth, suggesting that body size is the main pharmacokinetic determinant for children older than 2 years of age. Unfortunately, the present analysis did not include data from children younger than 1 year of age, most likely because children <1 year of age are commonly administered artemether-lumefantrine or quinine; thus, few studies have investigated the PK of artesunate-amodiaquine in infants <1 year of age. Due to the lack of available data for young children, the parameter values of the maturation function are influenced by information published for other drugs with CYP-dependent clearance. Other significant effects not included in our model may be relevant for very young children, including limited absorption capabilities.

Appropriate drug dosing in children is particularly challenging (48), and a number of studies have reported suboptimal dosing of children receiving antimalarial treatment (14,–19). Simulations presented in this analysis suggest that optimal plasma concentrations require doses of amodiaquine higher than those currently recommended for children weighing 8 kg, 15 to 17 kg, or 33 to 35 kg (Fig. 4). The same is true for patients weighing >62 kg. The proposed alternative dosing guidelines were developed on the basis of the strengths of currently available amodiaquine tablets, which are manufactured in strengths of 67.5 mg and multiples thereof. The amodiaquine dose adjustment proposed would increase complexity due to additional weight bands and would need to be implemented with suitable additional training and tools (Table 4). However, before routine implementation, the suggested regimen should be evaluated in prospective trials for efficacy and safety. Since amodiaquine and artesunate are often coformulated in a fixed-dose combination, the proposed dose changes would also increase the artesunate dose from 2.8 to 6.3 mg/kg/day to 3.6 to 7.4 mg/kg/day, which remains within the 2- to 10-mg/kg/day range recommended by WHO (49).

Simulations were used to propose optimized doses for the wide range of body weights recommended by the manufacturers for artesunate-amodiaquine administration (26). The simulated patients included very young children 2.5 to 15.2 months old with weights of 4.5 kg to <6.0 kg, which are less than those of any of the patients for which drug concentration-time data were available. For those patients, the proposed optimized dosages are based on the inclusion of allometric scaling and, even more critically, the maturation effect in very young children. Including these young children in the simulations could provide some understanding of the expected exposure in this group. Thus, the estimated exposure values and the suggested dose optimization in this subgroup of the patients should be treated as extrapolations and interpreted with caution. There is an urgent need to further investigate individuals in this age range.

The proposed new dosing recommendation suggests a substantial dose increase for particular patient groups compared to the currently recommended values; e.g., the dose for patients weighing 17 kg and 29 kg would change from 135 mg to 337.5 mg and 270 mg to 540 mg, respectively. As a consequence of the dose increase, these patients will experience higher peak concentrations, but the predicted median values of 560 ng/ml and 552 ng/ml for patients with weights of 17 kg and 29 kg, respectively, are within the range of peak concentrations found in other clinical trials of amodiaquine (49).

This analysis assumes that the concentration threshold for efficacy in the reference patient of 50 kg is equally applicable to younger individuals. However, the exposures required for efficacy in children may be higher than those required for efficacy in adults. This is because children tend to have lower levels of partial immunity in areas where malaria is endemic (50) and, consequently, higher parasite counts, lower hemoglobin levels, and a higher risk of treatment failure and progression to severe malaria. Yet, data on the safety of amodiaquine and any possible age dependence are limited. However, it will be necessary to collect safety data for this new dose before implementation. Further

studies of the proposed dosing schedule are thus needed to confirm safety and efficacy, especially in very young children.

As with all PK studies, there are limitations to the present analysis. Data for infants younger than 1 year of age were not available, thus resulting in uncertainty in the estimated maturation of clearance. Similarly, children younger than 12 years of age contributed a median of two samples per patient. The concentrations of amodiaquine were mostly below the LLOQ, making it difficult to characterize the pharmacokinetics of the parent compound in this age group. On the other hand, desethylamodiaquine concentrations were generally detectable, and thus, it was possible to obtain, even in this age group, a reasonably accurate description of the pharmacokinetics of the metabolite, which is the main source of pharmacological action. The description of pharmacokinetics in children was compensated for by including existing information on the maturation of the enzymes involved in metabolizing amodiaquine. This step made the estimates coherent with the general maturation profile (i.e., the enzymes are mature before 2 to 3 years of age), and it resulted in narrow bootstrap confidence intervals for the maturation parameters. Still, the results need confirmation. Precise information on the time of dose and/or the sampling time was not always available in some studies; hence, it was assumed that the times were consistent with the protocol schedule. In addition, different assays with different limits of quantification were used across the studies included in this analysis.

In conclusion, pooled individual concentration-time data for amodiaquine and its metabolite (desethylamodiaquine) were described using population PK modeling. Amodiaquine was described accurately by a two-compartment disposition model followed by a three-compartment disposition model for desethylamodiaquine. This study is the first to model the maturation of amodiaquine and desethylamodiaquine clearance as a function of postmenstrual age and, hence, provides the basis for further analysis of whether infants and young children achieve exposures equivalent to those in adults. The differences in amodiaquine PK between adults and children can largely be accounted for by body weight and the maturation effect. Amodiaquine exposures after standard daily oral doses were lower

in small children and in patients weighing more than 62 kg. The body weight-adjusted dosing regimen proposed in this study is expected to achieve similar exposure levels in all patients without an increased risk of acute toxicity.

3.5 Materials and methods

Clinical studies and data. This pooled analysis used data from previous studies conducted in different populations and across different geographical locations. Clinical and PK data from six studies (five cohorts) conducted in five countries (Burkina Faso, Ghana, Kenya, Uganda, and Thailand) were shared with the WorldWide Antimalarial Resistance Network (WWARN) and used for this analysis (Table 2). Relevant studies were identified by searching PubMed, Embase, Google Scholar, ClinicalTrials.gov, and conference proceedings, using the key words "amodiaquine pharmacokinetics" or "amodiaquine concentrations" and "clinical study." The first and last authors of relevant studies were contacted and invited to join this pooled analysis. Participating authors agreed to the WWARN terms of submission, which ensures that all data uploaded are anonymized and have been obtained with informed consent and in accordance with any laws and ethical approvals applicable in the country of origin (51). Information provided by each study included symptoms, dosing, drug concentrations, parasitemia, and clinical and laboratory data over time. Patients were administered either amodiaquine monotherapy or artesunate plus amodiaquine as a loose formulation or fixed-dose combination therapy. Treatment was given once daily for 3 days at a target dose of 10 mg/kg of body weight (Table 2). Data from 261 patients were pooled. Patients reflected a wide range of ages (1 to 60 years) and weights (6.5 to 93 kg). Only 26 pregnant women were included in the data pool. Detailed information on recruitment of study participants, randomization, and follow-up can be found in the reports of the respective studies (20,–25). Information on sample collection, storage, and the assays used is summarized in Table 2. Individuals were excluded from the analysis if information on drug dosage was unavailable, while protocol times were imputed for patients with missing information on the exact dosing times. All administered doses were converted to the amount of amodiaquine base (in milligrams) before modeling. The data sets for each study were merged and formatted for

subsequent analysis using Stata software (version 11; StataCorp, College Station, TX, USA) according to the Clinical Pharmacology Data Management and Statistical Analysis Plan (52, 53).

Structural model. Pharmacokinetic compartmental models were fitted to the observed concentration-time data for amodiaquine and desethylamodiaquine using nonlinear mixed-effects modeling with NONMEM software (version 7.3; Icon Development Solutions, Ellicott City, MD). Amodiaquine and desethylamodiaquine concentration measurements were fitted using the first-order conditional estimation method (54) with the eta-epsilon interaction. The Perl-speaks NONMEM, Xpose (version 4.3.5), Pirana (55), and R (version 3.1.2) (56) programs were used for automation and diagnostics during the model-building process. Nested models were assessed by their objective function value (OFV), computed by NONMEM to be proportional to −2 times the log likelihood of the data. A decrease in the OFV of at least 3.84 points was considered a statistically significant difference with *P* equal to 0.05, when comparing two hierarchical models with one parameter difference (χ^2 distribution with 1 degree of freedom [df]).

The structural (base) model was established using the most densely sampled clinical trial from the pooled data. Subsequently, data from different studies were added one by one in order of data richness, and each time the model was fit to the new data, reassessed, and modified if necessary (57).

One-, two-, or three-compartment disposition models with first-order elimination were investigated for amodiaquine. First-order absorption models with and without lag time and transit compartment absorption (58) were tested to describe drug absorption. Thereafter, the parameters for the best-performing amodiaquine model were fixed to the final estimates, and the structural disposition model for desethylamodiaquine was investigated. Amodiaquine was assumed to be completely and irreversibly metabolized to desethylamodiaquine (7), and a molar conversion factor was included to account for the difference in molecular weight between the two compounds. One-, two-, and three-compartment disposition models with first-order elimination were evaluated for desethylamodiaquine. Finally, the amodiaquine and desethylamodiaquine concentrations were fitted

simultaneously and the model was reevaluated. Additionally, we tested a semiphysiological model with hepatic extraction, implemented as previously described (59), with the aim of describing both hepatic clearance and first-pass extraction using the single parameter of hepatic intrinsic clearance (12, 60).

Several approaches to handling values below the lower limit of quantification (LLOQ) were tested (61, 62), ignoring the data below the LLOQ (M1), imputing LLOQ/2 for the first value in a consecutive series followed by ignoring the subsequent data below LLOQ (M6), or applying a likelihood-based approach (M3). Model stability and the robustness of the parameter estimates, as well as model run times, were considered when deciding on the approach for handling data below the LLOQ. All data below the LLOQ were retained in simulation-based diagnostics, i.e., visual predictive checks (VPCs).

Effect of body size and age. The impact of body weight was evaluated by allometric scaling of all clearance (exponent of 0.75) and volume (exponent of 1) parameters for amodiaquine and desethylamodiaquine, considering the strong biological prior of this relationship (47). A maturation function was investigated to characterize the age-related changes in clearance (39, 63). The individually predicted value of clearance was obtained by combining both the effect of size (allometric scaling) and the developmental process (maturation function), as shown in Equation 1:

$$CL = CL_{TV} \cdot \left(\frac{BW}{BW_{TV}}\right)^{3/4} \cdot \frac{PMA^{Hill}}{PMA^{Hill} + PMA_{50}^{Hill}} \quad (1)$$

where PMA is postmenstrual age (gestational age plus postnatal age), PMA_{50} is the PMA at which 50% enzyme maturation is achieved, and Hill is a shape factor for the relationship. CL is clearance, CL_{TV} is the clearance for a typical patient, BW is the individual body weight, and BW_{TV} is the body weight of a typical patient.

The reason for using PMA for the maturation function is that maturation begins *in utero* (39) and at birth organs have already achieved a certain level of maturation. No information on the duration of

gestation for the individual children was available, so PMA was obtained by simply adding 9 months to the postnatal age, assuming no premature births. For ease of interpretation, the results are presented and discussed in terms of postnatal age for a baby born at term. As no data were available for children younger than 1 year of age (among the studies included), prior information was used to stabilize the parameters of the maturation function to physiologically plausible values, comparable to those used for other drugs (16, 40, 42,–44, 64). The Prior functionality in NONMEM was employed for this (65), assuming weakly informative priors (10% uncertainty) for the maturation curve and using values of 12 months for $PMA_{50}$and 3.5 for the Hill coefficient. These values are in line with the maturation profile of CYP450 enzymes (46) and generally in line with the maturation of most drug elimination pathways (47), so they were considered reasonable priors for amodiaquine, which is mainly metabolized by CYP2C8 (7,–9).

Stochastic model. A log-normal distribution was assumed for between-subject variability (BSV) and between-occasion variability (BOV) in the PK parameters. The unexplained residual variability was modeled using a combined additive and proportional error model.

Drug concentration data from the different studies were obtained from different laboratories and with assays characterized by different limits of quantification, which might confer a different precision in the quantification of the lower concentrations in the pharmacokinetic profile. To account for this difference, we attempted to estimate a separate residual error for each study, but this proved unstable and resulted in the data sets with sparser sampling schedules being assigned unrealistically small errors and thus being overfit. As an alternative, we decided to conservatively add 20% of the LLOQ of each specific study to the estimated additive error component for the samples obtained in that study (Table 2). The value of 20% was chosen to remain consistent with the error level threshold generally used by analytical laboratories to define the limit of quantification when assays are developed according to drug development regulatory standards (66).

Whenever amodiaquine or desethylamodiaquine concentrations were detectable in the predose samples, those observed values were used to initialize all disposition compartments in the model to account for the prior presence of that drug in the body.

Covariate analysis. Predefined covariates considered for inclusion, besides weight and age (discussed above), were sex, drug combination (amodiaquine administered alone or in combination with artesunate) and formulation (loose formulation or fixed-dose combination), and the plasmodium species. A disease effect and any possible effects related to the patient's improving condition with treatment were investigated by testing the total parasite count and the hemoglobin (HB) concentration both at the baseline and on the days after initiating treatment and exploring whether the PK parameters varied after the initiation of treatment. Only three patients (1.2%) had missing parasitemia at the baseline; for these patients, the median value for their respective study population was used instead. Since some studies reported hematocrit (HT) and not hemoglobin (HB), the latter was estimated using equation 2 (67).

$$\mathrm{HB} = \frac{\mathrm{HT} - 5.62}{2.60} \quad (2)$$

The effect of covariates on PK parameters was assessed by using linear or exponential models for continuous covariates and additive proportional models for categorical covariates and by using a stepwise forward inclusion ($P < 0.05$) and backward elimination ($P < 0.001$) approach (68).

Model assessment. Model development was guided by improvements in the OFV, using the likelihood ratio test, and inspection of goodness-of-fit and other diagnostic plots. VPCs (69) stratified by country and age group were produced using Perl-speaks-NONMEM (PsN) with 1,000 simulations from the original data set. Eta and epsilon shrinkages were used to assess the reliability of empirical Bayes estimates and the power to detect model misspecifications in goodness-of-fit diagnostics (70). A nonparametric bootstrap with replacement (n = 500) was used to evaluate the robustness of the parameter estimates of the final model.

Simulations. Monte Carlo simulations were performed using the final PK model to explore the expected exposure and to evaluate optimal dosing regimens across different body weights. To ensure that the model would reflect exposure in accordance with the dosing guidelines for a specific population, in particular, underweight malaria parasite-infected children, *in silico* patients were generated by using individual demographic values (age and weight) from the studies included in the current analysis, plus other historical data (n = 748) from malaria patients (71–73) and routine clinical monitoring data for 1,580 children collected from the Bagamoyo Research and Training Center, a branch of the Ifakara Health Institute in Tanzania (unpublished). Pooling all these data, we obtained a database with 2,600 *in silico* patients covering a wide range of ages (2.5 months to 71.1 years) and body weights (4.5 to 93 kg). It is important to note that the age range of the patients used for simulations included ages lower than those for the children used to develop the final PK model.

Simulations using the final population PK model were repeated 5,000 times for each individual *in silico* patient. Subsequently, 3,000 exposures in each 1-kg weight band (1-kg intervals) were randomly drawn from the simulated results.

Desethylamodiaquine concentrations on day 7 were used as a proxy for efficacy, since they have been reported to correlate well with clinical cure rates (74). However, no target concentration value associated with clinical success is available in the published literature. The current dosing recommendation (49) results in reasonable cure rates in adults, suggesting that the exposure in a typical 50-kg adult with malaria is sufficient for treatment success. Thus, the simulated day 7 plasma concentration of desethylamodiaquine in this typical patient was assumed to be the relevant target concentration associated with clinical treatment success. The proposed dosing regimen was developed so that 75% of the patients in each 1-kg weight band were predicted to have day 7 desethylamodiaquine concentrations that were at least 80% of the target concentration. Also, the predicted maximum concentration (C_{max}) of desethylamodiaquine was monitored to evaluate potential acute toxicity associated with increased dosing. The predicted 75th percentile of C_{max} in the

patient weight group with the highest concentrations after receiving the currently recommend dosing was selected as a target level for potential acute toxicity. This reference target value was compared with the simulated exposures with the proposed dosing guidelines. Safety was assumed if the peak concentrations seen with the proposed guidelines did not significantly exceed the values already experienced by some patients with the current dosing recommendations.

3.6 Supplementary material

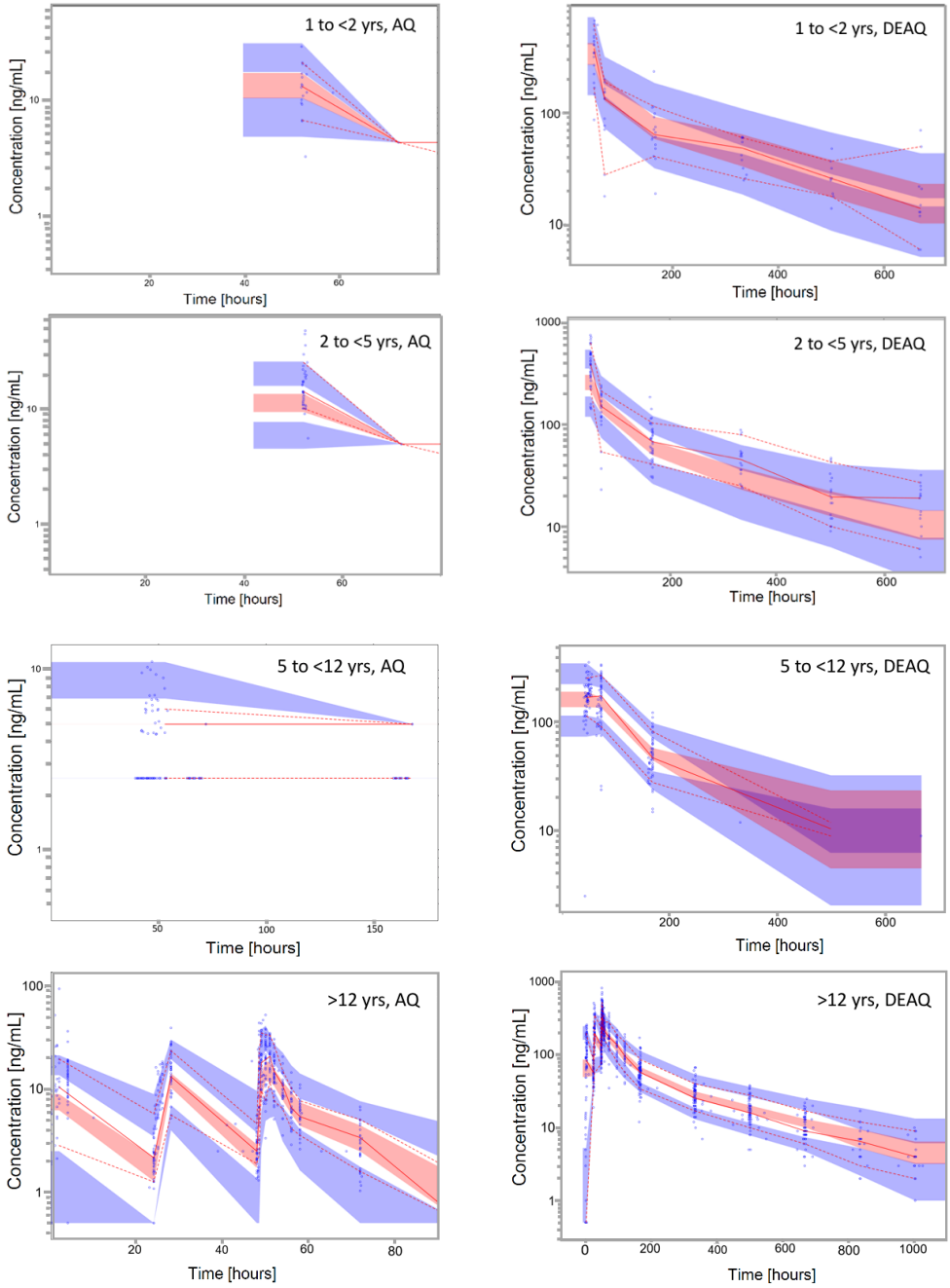

Figure S1 Visual predictive check of the final model describing the plasma concentrations of amodiaquine (AQ, left panels) and desethylamodiaquine (DEAQ, right panels) vs. time, stratified according to age groups. Open circles are the observed data points; solid and dashed lines are the 50th, 5th, and 95th percentiles of the observed data; shaded areas are the simulated (n=1000) 95% confidence intervals for the same percentiles. Plasma concentrations are shown on the log-scale. All observed and simulated values below the LLOQ have been imputed to LLOQ/2, using the LLOQ value specific to the study that contributed each observation. This is the reason for the horizontal percentile lines and thin confidence intervals visible in the panels referring to the lowest age groups. The VPC in the age group > 12 years for AQ was cut at 90 hours after the first dose since beyond this time the concentration of both observed and simulated data were below the LLOQ.

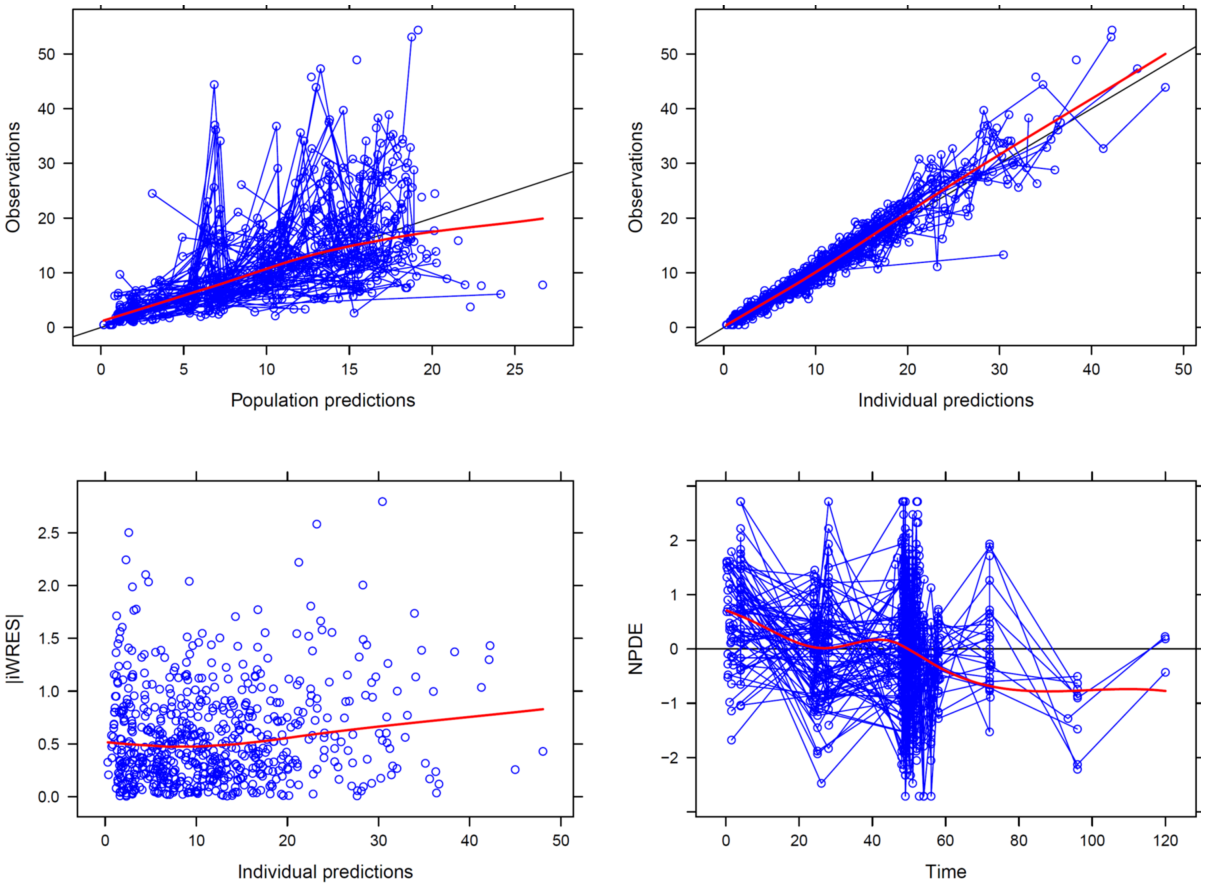

Figure S2A Basic goodness-of-fit diagnostic of the final population pharmacokinetic model of amodiaquine in uncomplicated *P. falciparum* malaria patients. Red lines, locally weighted least-squares regression; black lines, line of identity.

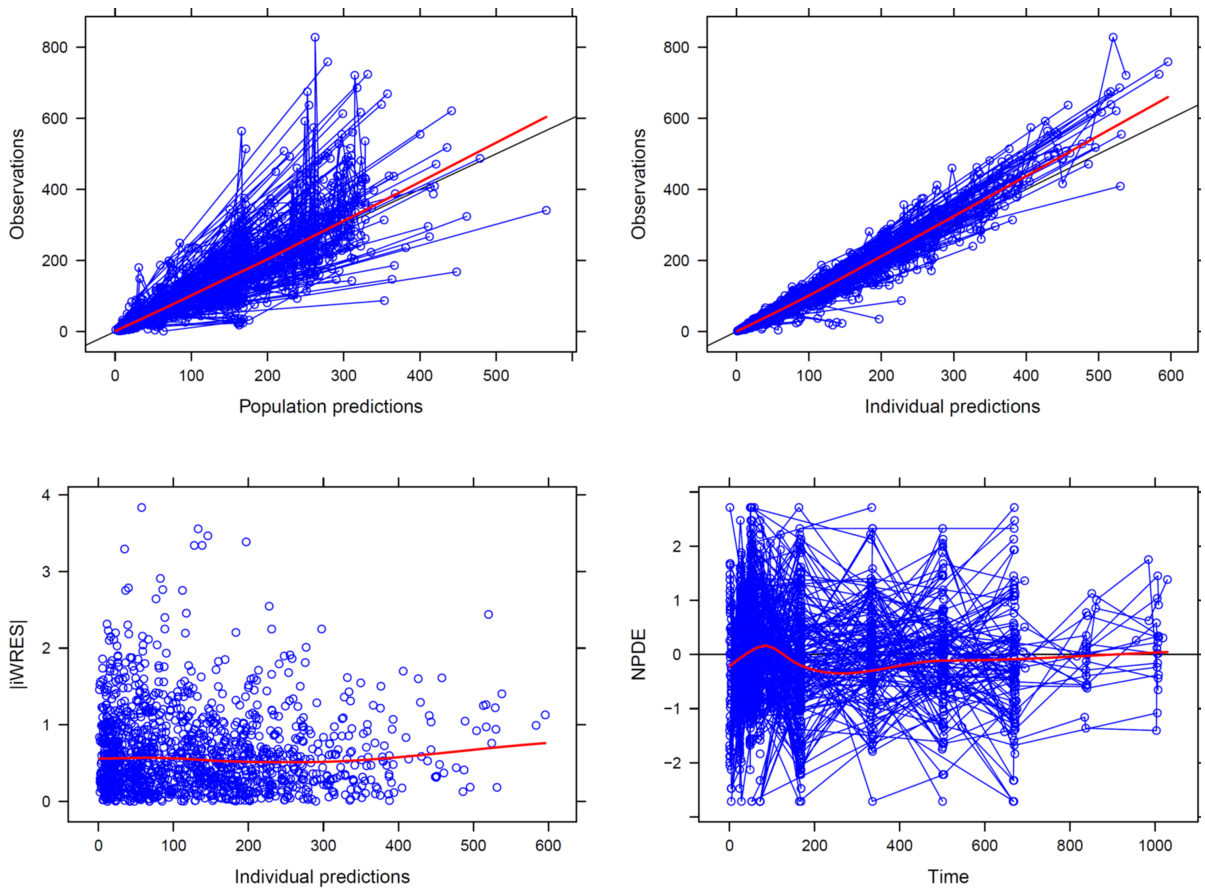

Figure S2B Basic goodness-of-fit diagnostic of the final population pharmacokinetic model of desethylamodiaquine in uncomplicated P. falciparum malaria patients. Red lines, locally weighted least-squares regression; black lines, line of identity.

3.7 Acknowledgements

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We all declare no conflict of interest.

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No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

3.8 Contribution information

for the WWARN Amodiaquine PK Study Group:

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Chapter four

4. Population pharmacokinetics properties of amodiaquine in Ghanaians children and adults with uncomplicated falciparum malaria using capillary whole blood samples

Working paper - analysis undertaken in the frame of a collaboration with WWARN and the Navrongo health research Centre. This publication is intended to be published in conjunction with clinical analysis and in collaboration with Dr. Oduro and Mr Anyorigiya. Intended to be published in Antimicrobial Agents and Chemotherapy Journal

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4.1 Abstract

The fixed formulation of amodiaquine and artesunate has been recommended as a second line treatment after artemether lumefantrine. However, there is limited information on the pharmacokinetic properties of amodiaquine in capillary whole blood. This study evaluated the population pharmacokinetic properties of amodiaquine and its active metabolite desethylamodiaquine from capillary whole blood collections from 242 Ghanaian adults and children with uncomplicated Plasmodium falciparum malaria. Nonlinear mixed effects modelling was used to model concentrations over time of both amodiaquine and desethylamodiaquine. A two-compartment model best described the disposition of both amodiaquine and desethylamodiaquine. Similar to previous analysis, clearance was found to mature with post menstrual age with children reaching 50% of adult clearance levels at 6 (95% CI: 4.1–8.2) and 2.3 (95% CI: 1.3–3.0) months after last menstrual period for amodiaquine and desethylamodiaquine, respectively. Bioavailability was found to be 14% lower for each unit increase in baseline haemoglobin value. Elimination clearance of amodiaquine was estimated as 9% lower with each log-increase in baseline parasitemia. In summary, we have investigated age maturation effect on amodiaquine clearance by including children age less than one year and confirmed the age maturation effect on clearance found in a pooled analysis of amodiaquine and desethylamodiaquine. Further research should investigate the population pharmacokinetics of amodiaquine when both venous plasma and capillary whole blood are used to enable full comparison and prediction of drug exposures from different sampling matrices.

4.2 Introduction

Plasmodium falciparum malaria remains a concern globally with about 3.2 billion people at risk of infection in 2015 (1). The estimated number of annual malaria cases in 2015 caused by *P. falciparum* was 212 million, with about 429,000 malaria deaths. Of all malaria deaths, 70% were in children under five years of age (1). Currently the World Health Organization (WHO) has recommended five artemisinin-based combination therapy (ATCs) for the treatment of *P. falciparum* malaria. Artesunate-amodiaquine (AS-AQ) is currently recommended by WHO as the second treatment line after artemether-lumefantrine (2). Artesunate (AS) is responsible for the initial fast killing of malaria parasites in treated patients, only remaining in the body for a short time due to the rapid absorption and short half-life of artesunate (3, 4). The partner drug amodiaquine is metabolized to desethylamodiaquine (5, 6), an active metabolite with a long elimination half-life (7, 8) and is responsible for the remaining anti-malarial activities. After oral administration amodiaquine is rapidly absorbed and metabolized in the liver to desethylamodiaquine by the cytochrome P450 isoenzyme 2C8 (7, 9). The protein binding of amodiaquine is about 90% (8) and α_1-acid glycoprotein is the main binding site (10).

Several population pharmacokinetic (PK) modelling studies have been conducted for amodiaquine using venous plasma from participants (11–16). However, population PK analysis on whole blood capillary samples of amodiaquine has been limited (17). Different matrixes for PK samples can yield different estimates of the population PK parameters. desethylamodiaquine has an estimated whole blood to plasma ratio of 3:1 in healthy volunteers (5) and 0.8:1 in malaria patients (18).
More recently a pooled PK analysis, Ali *et al*. 2018 (16) found age maturation effect on the clearance of amodiaquine and desethylamodiaquine. In this study no data were available for children below 1 year of age and hence the estimation of maturation parameters is driven by prior information (Ali et al. 2018).

The main objective of the present analysis was to assess the population PK of amodiaquine and its active metabolite using non-linear mixed effects modelling of whole blood capillary PK samples taken from both adult and children malaria patients with clinical symptoms. In particular, to assess PK in children under one year of age and to investigate the age maturation effect on clearance in these patients. Furthermore, we aim to assess the factors affecting PK parameters of amodiaquine from whole blood capillary matrix compared to those found using plasma matrixes.

4.3 Methods

Clinical study and data. The previously reported PK clinical study was conducted inside Navrongo municipal in Ghana from August 2011 to February 2012 and again from July 2012 to January 2013 (19). This PK study was part of a clinical and safety study in which detailed information on how subjects were recruited, randomized and followed up have been reported before (19). In short, the study involved children from one months through to adults 50 years of age who attended the War Memorial Hospital in Navrongo with uncomplicated P. falciparum malaria to seek treatment. The study was conducted following approval from the Ghana health service ethics review committee, the Navrongo Health Research Centre ethics review board and the University of Cape Town human research ethics review committee. Informed consent from each participant was obtained (19).

Patients were administered with artesunate-amodiaquine combination therapy in a fixed dose formulation daily for 3 days. All patients were followed up to 28 days. PK samples were collected before administration of each dose on day 0, 1 and 2 and then on day 3, 7, 14 and 28. Capillary whole blood samples were taken from all patients. Extensive details on how the sample were separated, stored, quantified and analyzed can be found elsewhere (19).

Data analysis and Pharmacokinetic modeling. Data management and summary statistics were completed using Stata (version 13, College Station, Texas, USA), and individual plots constructed using R version 3.1.2 (20) software to identify extreme observations. Compartmental PK models were fitted to the data using NONMEM (linear mixed-effects modeling, Icon Development Solutions, Ellicott City, MD) version 7.3 with first-order conditional estimation method (FOCE) with interaction (21).

Amodiaquine was assumed to be completely metabolized to its active metabolite (22). Elimination of both amodiaquine and desethylamodiaquine was assumed to take place from the central compartments. Models were fitted simultaneously for amodiaquine and desethylamodiaquine and different disposition models (one-, two- or three-compartment) with first order absorption models investigated. The first order absorption rate (Ka) were fixed to values obtained from previous pooled pop-PK analysis (16) because limited information could be obtained from this present study on absorption phase as there were no PK samples between 0-24 hours. The delay in drug absorption for amodiaquine was explained by lag time fixed to the corresponding value obtained from the pooled PK-analysis (16).

Allometric scaling (23) based on body weight (BW) for clearance (equation 1) and volume of distribution (equation 2) parameters for both the parent and metabolite was implemented to scale for size, namely:

$$F_{size} = \left(\frac{BW}{BW_{std}}\right)^{3/4} \quad (1)$$

and

$$F_{size} = \left(\frac{BW}{BW_{std}}\right)^{1} \quad (2)$$

Where: BWstd is the weight of the standard patient.

To characterize changes in clearance, individual clearance estimates were further normalized through an age-related maturation model using a Hill model (24) on amodiaquine and desethylamodiaquine clearance. To ensure parameter estimates for the maturation function were stable and remained at physiological plausible values, prior information was used. The value of the postmenstrual age at 50% maturation (PMA_{50}) and the Hill coefficient were obtained from a previous study on enzyme CYP2C9 (25) to be used as priors, both with 10% uncertainty in a lognormal distribution. CYP2C9 is similar to the amodiaquine metabolizing enzyme (7, 26, 27).

A log-normal distribution for between-subject (BSV) and occasion variability (BOV) was assumed. Between occasions variability was assumed to affect only bioavailability.

Goodness-of-fit assessment during modeling was undertaken using Perl-speaks NONMEM implemented in NONMEM (PsN; version 3.2.4), and Xpose version 4.3.5 (28) in RStudio (29). Hierarchical models were compared by their objective function value (OFV), and a decrease in OFV of at least 3.84 points (p = 0.05, χ^2 distribution with 1 degree of freedom) was considered to be a statistically significant difference (30). To assess whether the addition of covariates (such as sex, hemoglobin, parasitemia etc.) influenced the PK parameters of both amodiaquine and desethylamodiaquine, individual estimates of random effects (etas) obtained from the model without covariates were plotted against individual values of each covariate to visualize the potential relationship. Etas that varied with covariates were included in the model and covariate selection was based on change in OFV, a drop of OFV > 3.84 was considered significant at the 5 % level for an addition of one covariate into the model. Backward deletion of covariates was carried out and only physiological plausible covariates were kept if associated with an increase in OFV of > 10.83 (0.1 % significant level) on their removal (30). Covariates tested for inclusion were age, baseline parasitemia, baseline hemoglobin (HB) and sex. Linear or exponential models were assessed for continuous covariates and additive proportional models for categorical covariates.

Model development was guided by improvements in the objective function value (OFV) and inspection of goodness of fit plots and VPCs (31). A non-parametric bootstrap with replacement (n = 1000) was used to evaluate the robustness of the parameter estimates of the final model.

4.4 Results

A total of 242 malaria patients were available for analysis (19). One hundred and eleven (45.9%) were male and 147 (60.7%) were children under five years of age. The median (range) age was 4 (0.1 – 49.7) years and the median (range) weight was 15 (6.5 – 64.0) kg. The geometric mean (range) baseline parasitemia was 28726 (1080 – 204480) parasites/µL of blood. Baseline values of the study population are presented in Table 4.1 and were reported previously (19).

A total of 1378 post-dose capillary whole blood samples for both amodiaquine and desethylamodiaquine were available for population PK analysis. A total of 508 (36.9%) post-dose amodiaquine samples and 18 (1.3%) desethylamodiaquine samples were below the limit of quantification (BLOQ). Seventy-one (5.4%) amodiaquine samples and 81 (6.1 %) desethylamodiaquine samples were removed from analysis because their values were deemed biologically implausible following investigation of individual plots. The lower limit of quantification (LLOQ) value was 0.781 for amodiaquine and 3.91 for desethylamodiaquine.

Table 4.1 Baseline characteristic of the population

Parameter	
Sex - n (%)	
Female	54.1% (131/242)
Male	45.9% (111/242)
Median (range) age (yr)	4.0 (0.1 – 49.7)
Age in years	
< 1	4.6% (11/242)
1 - < 5	56.2% (136/242)
5 - < 12	31.8% (77/242)
12+	7.4% (18/242)
Nutritional status - n (%)	
Below 2SD	14.7% (21/143)
Between 3 SD and 2 SD	11.2% (16/143)
Below 3 SD	3.5% (5/143)
Enrolment vital and laboratory parameters	
Median (range) weight (kg)	15.0 (6.5 – 64.0)
Median (range) temperature (°C)	38.1 (36.0 - 40.2)
Geometric mean (range) parasitaemia (parasites/µL)	28,726 (1,080 – 204,480)
Median (range) haemoglobin (g/dL)	10.2 (5.6 - 16.6)
Median (range) dose (mg/kg)	33.1 (23.0 – 45.0)

Amodiaquine and its metabolites were simultaneously modelled using Nonlinear Mixed Effect Modelling. A two-compartment model best described the disposition of both amodiaquine and desethylamodiaquine, with a first order absorption via a lag-time which was fixed to 0.23 hours. The absorption rate constant was fixed to 0.59 1/h (182) for amodiaquine. The structural final model is depicted in Figure 4.1. Baseline patient's body weight was used to allometrically scale all clearance and volume. All clearance and volume of distribution parameters were scaled by body weight of 15 kg. Inclusion of body weight as an allometric function in all clearance improved the model and was associated with an OFV drop of 233 points, while for all volume of distributions, the OFV drop was 118 points. Effect of maturation on amodiaquine clearance (CL) and desethylamodiaquine clearance (CL-DEAQ) improved the model by 234 and 155 points, respectively.

Clearance matures with postmenstrual age (Figure 4.3). Prior information on the postmenstrual age at which 50% of adult maturation (3 months after births) and the hill coefficient of 3 were used (127) both with 10% uncertainty in a lognormal distribution to constrain them to a physiologically plausible value.

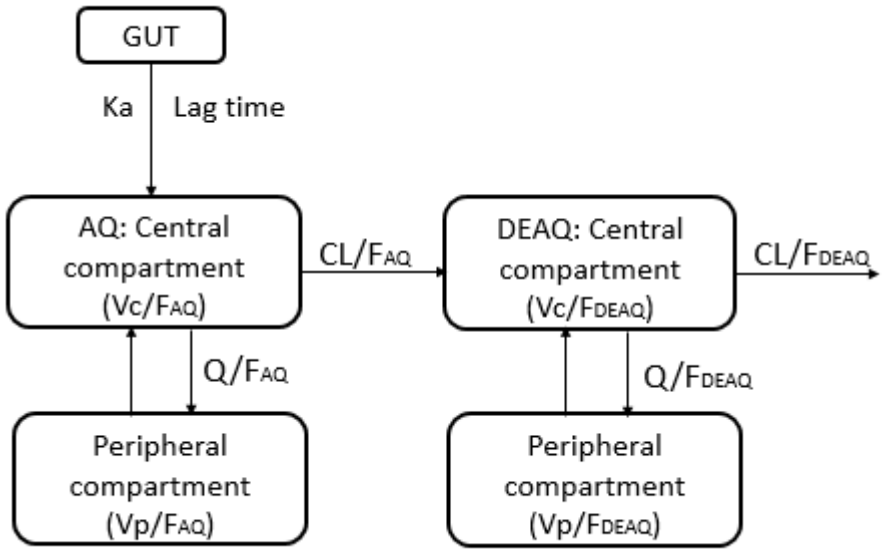

Figure 4.1 Schematic diagram of the final structural model describing amodiaquine and desethylamodiaquine population pharmacokinetics in malaria patients in Navrongo Ghana. Amodiaquine is absorbed to a central amodiaquine compartment (VC/FAQ) via lagged first-order absorption rate (K_a) and distributed into peripheral compartment (VP/FAQ). The eliminated amodiaquine (CL/FAQ) from the central compartment forms desethylamodiaquine (central desethylamodiaquine compartment (VC/FDEAQ)). Desethylamodiaquine is distributed into peripheral compartments (VP/FDEAQ) and then eliminated (CL/FDEAQ). Abbreviations: CL = clearance, V = volume of distribution, Q = inter-compartmental clearance, AQ = amodiaquine, DEAQ = desethylamodiaquine.

An enzyme maturation function resulting in 50% maturation at 6 (4.1–8.2) and 2.3 (1.3–3.0) months after birth (assuming standard baby born at term) for amodiaquine and desethylamodiaquine respectively. Adding an effect of hemoglobin on bioavailability dropped the OFV by 210 points (p-value = < 0.001, 1 degree of freedom). Bioavailability was found to decrease by 14.2% (95% CI: 10.2 – 18.4%) for each 1 g/dL increase of hemoglobin value. Including a parasitemia effect on clearance improved the model significantly (ΔOFV = - 192, p-value < 0.001, 1 degree of freedom). Clearance was decreased by 8.81% (95% CI: 4.39 - 13.2%) for each increase of parasitemia/µL (in a log-scale). The effect of treatment occasion on bioavailability was not significant. The parameter estimates of the final model together with their precision obtained with a non-parametric bootstrap replacement (n = 1000) are given in Table 4.2.

Visual predictive checks (VPCs) plots show that the model adequately fitted the data (Figure 4.2). However, after adjusting for all the factors above, the model still detected a large (> 60%) random variability in the bioavailability and BSV in the peripheral compartment parameters for amodiaquine and in clearance of central compartment for desethylamodiaquine.

Table 4.2 Final parameter estimates of the population pharmacokinetic model for amodiaquine and desethylamodiaquine in malaria patients

	Original data	Bootstrap: 95 % CI
Parameter[a]	Estimates[b]	Estimates[c]
Amodiaquine		
Ka [1/h]	0.59 Fixed	-
LAG	0.23 Fixed	-
F	1 Fixed	-
CL/F [liters/h]	171	130, 217
Vc/F [liters]	1850	1210, 2940
Q1/F [liters/h]	20.4	12.5, 29.9
Vp1/F [liters]	4050	2420, 6340
Additive error	0.242	0.210, 0.284
Proportional error (%)	41.3	0.368, 0.474
Desethylamodiaquine		
CL/F [liters/h]	3.06	1.47, 4.14
Vc/F [liters]	633	536, 716
Q/F [liters/h]	2.35	1.57, 3.44
Vp/F [liters]	2910	777, 8230
Additive error	14.7	10.3, 20.7
Proportional error (%)	31.9	0.283, 0.346
Covariate effects		
PMA50 for AQ [months]	6	4.1, 8.2
Hill for AQ []	3.16	2.87, 3.41
PMA50 for DEAQ [months]	2.3	1.3, 3.0
Hill for DEAQ []	3.53	3.18, 3.70
Hemoglobin effect on F (% reduction)	14.2	10.2, 18.4
Parasite density effect on CL (% reduction)	8.81	4.39, 13.2
Interindividual variability (% CV)		
CL/FAQ [liters/h]	0.092 (30.4)	24.5, 38.6
Q/FAQ [liters/h]	0.610 (78.1)	64.4, 88.6
Vp/FAQ [liters]	0.464 (68.1)	2.04, 83.1
CL/FDEAQ [liters/h]	0.548 (74.0)	46.6, 149.0
Vc/FDEAQ [liters]	0.116 (34.1)	17.5, 45.8
F	0.382 (61.8)	47.2, 73.4
Interoccasion variability		
F	0.403 (63.5)	50.1, 75.0

[a]AQ, amodiaquine; DEAQ, desethylamodiaquine; Ka, absorption rate constant; F, relative bioavailability; CL/F, oral clearance; Vc/F, central volume of distribution; Q/F, intercompartmental clearance; Vp/F, peripheral volume of distribution; PMA50, time to reach 50% maturation; Hill, steepness of the maturation curve.
[b]Computed population mean parameter estimates from NONMEM are calculated for a typical patient with body weight of 15 kg. The root mean square error (% RME) calculated as (standard deviation/mean value) X 100.
[c]Computed from nonparametric bootstrap method of the final model (n = 1000).
The coefficient of variation (% CV) is calculated as (estimate)^{½} X 100.

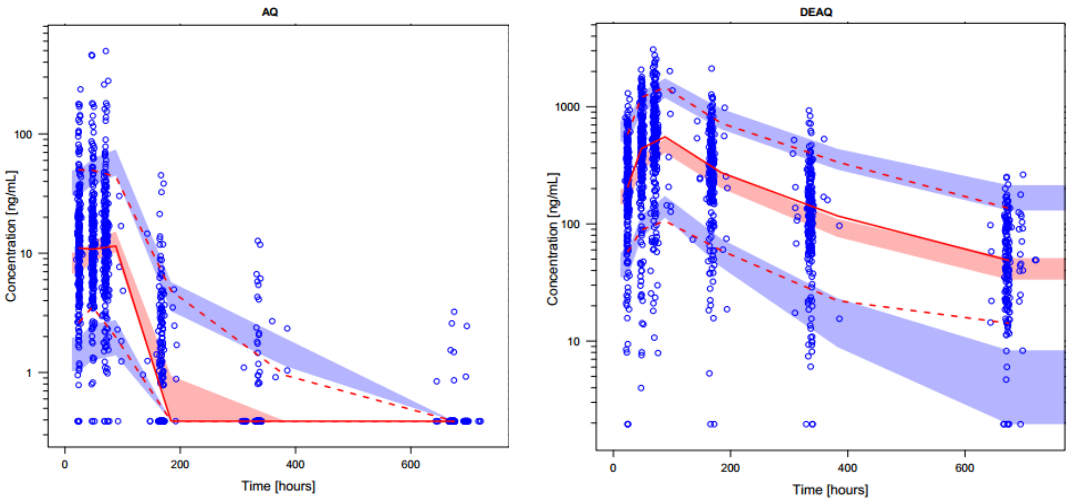

Figure 4.2 The Visual predictive check of the final model (left amodiaquine and right desethylamodiaquine). The open circles represent the observations; solid and dashed lines represent the 50th, 5th, and 95th percentiles of the observed data; shaded areas represent the simulated (n=1000) 95% confidence interval around the 50th, 5th, and 95th percentile. Y-axis represent capillary whole blood concentration on the log-scale.

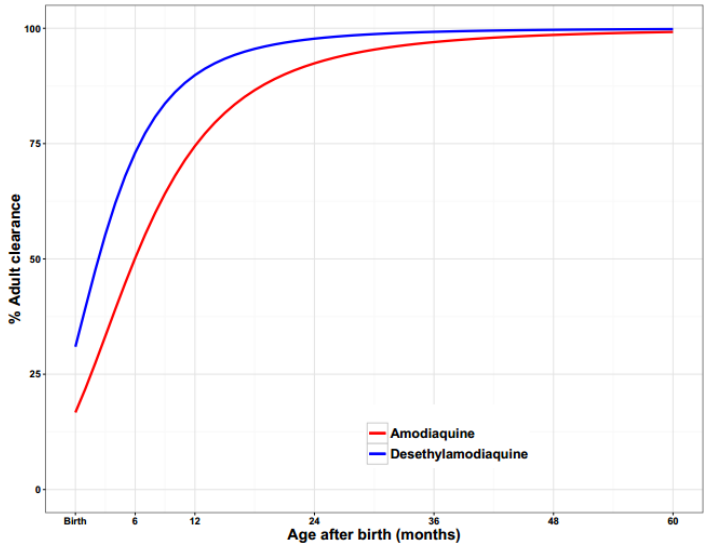

Figure 4.3 Predicted age maturation effect of clearance of amodiaquine (red line) and desethylamodiaquine (blue line) expressed as a fraction of adult clearance plotted against age after birth (assuming birth occurred at term).

4.5 Discussion and conclusion

Amodiaquine in fixed formulation with artesunate has been recommended by WHO for the treatment of uncomplicated malaria and is currently in use in many malaria endemic countries of Sub-Saharan Africa (2). This study developed population pharmacokinetics of amodiaquine from capillary whole blood concentrations. Two-compartment models best described both amodiaquine and desethylamodiaquine, with a lag time in the absorption phase. Apart from the effect of body weight on all clearance parameters (scaled allometrically to power ¾) and on all apparent volume of distribution parameters (scaled allometrically to power 1), the model also detected age maturation effects on amodiaquine and desethylamodiaquine, effects of baseline hemoglobin on oral bioavailability, and effects of baseline parasite density (parasites/µL) on amodiaquine clearance.

The two-compartment model for amodiaquine is in line with results from the recent pooled analysis by Ali Ali *at al*. 2018 and by Joel *at al*. 2012 in Asian pregnant women (12). The counterpart non-compartmental analysis of this study revealed a biphasic elimination (similar to a two compartmental model) for amodiaquine (19). Several drugs of the quinolone family were also best described by two compartmental models (32–37). Overall there is apparent consistency across studies for describing amodiaquine with a two compartmental model. However, there are several earlier studies in African populations that described amodiaquine disposition by one compartmental model (11, 14).

A two-compartment model for desethylamodiaquine kinetics is in contrast to the pooled analysis of Ali Ali *at al*. 2018 and a study in pregnant women in Asia (12) where a three-compartment model was found. Our study revealed the same structural model to the studies that found one compartment models for amodiaquine (11, 14) and is in line with non-compartmental analysis counterpart (with biexponential elimination) (19). Unlike our study and the other studies in African populations where patients were followed up to maximum of 28 days, the study in Asian pregnant women were followed up to 42 days and more samples were taken. More frequent sampling with longer duration of follow up may explain the use of a more complex structural model.

The whole blood to plasma ratio for amodiaquine was found to be different depending on health of a patient, being 3:1 for healthy subjects (5) and 0.8:1 for malaria patients and increasing linearly to 3:1 when the patients became malaria free (18). Therefore, parameters estimate in this study should not be compared directly to the studies where sample matrices are not the same. The pharmacokinetic parameters in our study, however, followed the same pattern as in the previous pooled analysis, showing large interindividual variability and large apparent volume of distribution Table 4.2 (16). More variability in the capillary samples is expected as they were taken from a finger

prick where pressure on the finger may have been applied before the sample was taken, thus resulting in vary amounts of interstitial fluid.

The first sample in this study was collected 24 hours after the first dose and only one sample was collected after the first and the second dose. Because we did not have strong information in the absorption phase of this drug, the rate of absorption as well as the lag time were fixed to values found from the pooled analysis of the same drug (16). The values were deemed appropriate as they were derived from a population model with a large number of patients and samples and hence estimated with high precision. In addition, the higher between occasion variability in bioavailability found in this study could be explained by the sparse information in the absorption phases (after the first two doses). We also observed higher variability in the peripheral compartment in this study, potentially explaining the higher proportion of below limit of quantification for amodiaquine (35.4%) in the later samples (which explain the bio-phase of the AQ concentration-time profile).

Our study had a large number of patients ranging from 0.1 – 49.7 years and weight 6.5 – 64.0 kg and significant samples per patients giving higher power to detect covariate relationships. Body weight allometrically scaled to both elimination clearance and volume of distribution were the most significant and important covariates in our study. This was also reported in the pooled-analysis for amodiaquine involving both children adults (16).

Inclusion of age maturation effects on clearance of both amodiaquine and desethylamodiaquine significantly improved the model fit. Confirming that allometric scalling with body weight alone cannot sufficinently explain that metabolic enzymes mature during the first year of life, namely that at birth CYP-dependent metabolism is about 50% to 70% of adult's levels (24, 38, 39). Here, clearance at birth was estimated to be 17% and 31% of adult clearance for amodiaquine and desethylamodiaquine, respectively. In addition, children were determined to reach 50% of an adult clearance value at 6 months of age for amodiaquine, and 2.5 months of age for desethylamodiaquine and the 100% values at about 4 years of age. This finding is in agreement with results from a pooled analysis of amodiaquine (16) and other drugs (38, 40–44). However, prior information was used to constrain values of the maturation function to physiologically plausible values reported previously (38, 40–44).

Another important covariate relationship was the effect of hemoglobin level (HB) on bioavailability, resulting in higher exposure of amodiaquine in more anemic children. This was in addition to inclusion of both allometric scaling and maturation effect on clearance. Age and HB were significantly correlated in this population ($\rho = 35\%$, p-value < 0.001), with younger children tending to have lower HB level. Both bioavailability and clearance are the predictors of area under the curve (exposure) and thus could explained this finding. However, removing HB the model fit declined both in terms of OFV ($\Delta OFV = 34.7$) and VPCs. A non-compartmental analysis from this data (19), revealed

higher maximum concentration (C_{max}) for patient with HB less than 8.0 g/dL. Additionally, Batty *et al.* 2012 (45) found that for an antimalarial of the same family as amodiaquine (46), for every 1 g/dL increase in HB the apparent volume of distribution of naphthoquine increased by 16%. The lower bioavailability in patients with higher hemoglobin level in our study reflects patients with lower values of volume of distribution appearing to contradict the finding from Batty *et al*. 2012.

Clearance was estimated to be 9% lower with each log-increase in initial parasitemia, implying that patients with higher parasitemia resulted in higher exposure as measured by the area under the curve (AUC). This is in line with a non-compartmental analysis of this data, reporting significantly higher elimination half-life for desethylamodiaquine in patients with higher (≥ 100,000) parasites/µL (19), equivalent to lower clearance of desethylamodiaquine in our analysis. A 2-fold reduction in systemic clearance in malaria patients compared to healthy subjects when evaluated with non-compartmental analysis (47) was observed. However, contrasting these results, Winstanley *et al* 1990 reported higher AUC for amodiaquine in healthy volunteers than in malaria patients (5, 18). Higher AUC may imply lower elimination clearance or higher bioavailability or both. Joel *et al* 2012 found relative bioavailability for dihydroartemisinin was 28% higher with each log-increase in initial parasitemia (48), Indicating higher AUC for patients with higher parasitemia. Amodiaquine is mainly metabolized by hepatic biotransformation (7, 22, 27) and the lower clearance in patients with higher parasitemia can be attributed to the reduction in liver blood flow or the impairment of the hepatic elimination capacity (8).

Higher AUC for amodiaquine in young children was found in a previous non-compartmental analysis of this data (19) which is similar to our population PK analysis. The age maturation effect on clearance found in our study could in part explain the higher AUC in young children in the non-compartmental analysis (19). In addition, children less than one year of age were determined to have 74% of adult clearance for amodiaquine. A higher elimination half-life for amodiaquine in the non-compartmental analysis was found in children less than one year as compared to older children (19) explaining the clearance maturation with age found in our study. Additionally, relative bioavailability was estimated to be14% lower for each 1 g/dL increase in hemoglobin level and can explain the higher AUC in young children in the non-compartmental analysis (19).

An effect of weight for age Z-score on volume of distribution of amodiaquine, effect of sex on C_{max} and clearance of desethylamodiaquine, and effect of dose in mg/kg AUC of desethylamodiaquine was also found in a previous non-compartmental analysis (19). These factors were directly or indirectly taken in to account through the use of allometric scaling with body weight and maturation effect in our population PK analysis.

This study has some of limitations. Firstly, no data were available during the absorption phase and thus use of parameters estimates from a previous pooled analysis of venous samples could have influenced our analysis. Other study limitations include the limited number of malnourished children, lack of data with other co-morbidities, and no data on pregnant women with malaria. Thus, the pharmacological properties of amodiaquine in these populations could not be assessed reliably or not at all. Prospective pharmacological studies are needed to address potential differences in these sub-population.

In conclusion, the population pharmacokinetic model fitted to amodiaquine and its metabolite (desethylamodiaquine) from capillary whole blood concentration time data found that two compartments best described both amodiaquine and desethylamodiaquine. This study confirmed the effect of maturation on amodiaquine and desethylamodiaquine clearance found in a pooled analysis of amodiaquine (16) and that, the results are in agreement with the non-compartmental analysis previously completed on the same study cohort (19). Additional factors affecting pharmacokinetic properties of amodiaquine were also found in this analysis which were not found before for amodiaquine. We have found that, hemoglobin and malaria parasitemia affect parameters (bioavailability and clearance) of exposure (AUC), and thus dose adjustments should be investigated for young patients (who generally have lower hemoglobin and higher parasitemia during malaria illness). Population pharmacokinetic analysis on patient data with both venous plasma and capillary whole blood is needed to directly compare the parameters of PK model and to be able to predict drug exposures from different sampling matrices.

4.6 Acknowledgments

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We all declared no conflict of interest.

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Chapter five

5. Population pharmacokinetics of antimalarial Naphthoquine in combination with Artemisinin in Tanzanian children and adults

Working paper - analysis undertaken in the frame of collaboration with Medicine for Malaria Venture (MMV) and the Ifakara Health Institute (IHI). This publication intended in conjunction with safety analysis and in collaboration with Mr Said Jongo (MD) and Dr Nathalie Gobeau. Intended to be published in Antimicrobial Agents and Chemotherapy Journal

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5.1 Abstract

The combination antimalarial therapy of artemisinin-naphthoquine (ARCO) has been developed as a single dose therapy. This is expected to improve adherence relative to 3-day (or longer) schedules for other artemisinin combinations. Here the population pharmacokinetics of naphthoquine in adults and children over 5 years of age with uncomplicated malaria in Tanzania is described and used to assess levels of exposure achieved with current dosing recommendations. Twenty-nine patients were dosed with median dose of 7.4 (IQR: 6.7-7.5) mg/kg of naphthoquine and plasma concentrations assessed at 13 time points after dose over 42 days. Pharmacokinetic compartmental modelling using nonlinear mixed-effects was used to characterize the plasma concentration-time profiles. The distribution phase of Naphthoquine was best described by a three-compartmental model with ten transit compartments describing the absorption phase. Artemisinin distribution was best described by a one compartmental model and 13 transit compartments for absorption. From simulation, children weighing 29 – 32 kg, and patients of 46 – 49 kg and > 63 kg were estimated to have received on average 25% lower day 7 concentration average compared to a 50 kg patient. This suggests a dose increase for some weight groups in young children and adults will ensure adequate exposure. Furthermore, use of the final PK model to explore naphthoquine exposure obtained with multiple doses of ARCO, indicates multiple doses will potentially provide beneficial curative outcomes compared to single dose and would also be in line with current recommendations for antimalarial including artemisinin.

5.2 Introduction

There is urgent need to assess existing antimalarials, as well as to develop novel antimalarials, to address observed declines in efficacy in some artemisinin-based combination therapies (1) and drug resistance. At present artemisinin resistance for *Plasmodium falciparum* is present on the Thai-Cambodia border (2–5) and spreading or emerging in African settings (6). The treatment course of current artemisinin-based combination therapies (ACTs) is based on a recommended 3-day regimens to ensure sufficient artemisinin exposure, but exposure might be compromised by poor patient adherence (7–10). Adherence is thus thought to be a factor in the development of drug resistance (11). To improve patient adherence, a single dose therapy would be preferable to the 3-day regimen, and an oral single dose regimen has been developed for the combination of artemisinin and naphthoquine phosphate (ARCO), a new generation ACT (12). However, despite the improved adherence with single dose therapy, cure rates with ARCO may improve with multi-day dosing (13–14). There is a need to assess both the efficacy and pharmacokinetics of different regimens of ARCO.

Artemisinin has a fast acting parasiticidal action (15–16), but because of its very short circulating half-life, when used alone it has the disadvantage of high recrudescent rates (17) and risk of drug resistance (18, 19). Naphthoquine on the other hand, has a longer circulating half-life (11, 20) and higher cure rate (21), though it has slower onset of parasite killing (22). Combining these two drugs have an advantage of overcoming the individual weakness and hence reduce the pressure of drug resistance. Artemisinin reduce the parasite number very rapidly and the residual parasites is then exposed to relatively high levels of partner drug (23) with longer elimination half-life.

The current ARCO recommended dosage for adult is a single dose containing 1000 mg artemisinin and 400 mg naphthoquine. Doses for children has been based on the adult dose adjusted via body weight (22). As previously reported for several drugs (24–29), dose adjustment for children based on body weight derived from adult's population (30) may result in inappropriate exposure levels of ARCO in children. However, for Coartem similar exposure between adult and children dosed based on body weight was observed (31).

In Tanzania, at the time of the study, ARCO was registered by Kunming pharmaceuticals, Kunming, China and had been used for treatment of uncomplicated malaria for patient of all ages including young children. However, the pharmacokinetic properties have not been studied among this Tanzanian population nor in young children. Extrapolating pediatric dose regimens from data on adults has resulted in substantial under dosing of children for several drugs (24–29). For malaria this may result in lower exposure and malaria recrudescence, for example in a study conducted in Burkina Faso, young children (2 – 5 years) received lower exposure compared to older children (6 – 10 years) after receiving piperaquine doses based on allometric scaling (32).

The safety and efficacy of ARCO has been assessed in several clinical studies (22), however, no study has been completed to assess exposure in Tanzanian population, nor to assess population-PK in this population. A study was undertaken to confirm safety, tolerability and efficacy of ARCO in a Tanzanian setting, as well as its pharmacokinetic properties. The objective of the present analysis is to develop a population pharmacokinetic model of naphthoquine in a Tanzanian population, and thus assess if the current dosing in children results in similar exposure levels to adults. Furthermore, the resulting model used to thus explore alternative optimal dosage regimens via simulation, for both a single and multi-dose option.

5.3 Methods

Study area and design. Pharmacokinetics (PK) data was obtained from an artemisinin-naphthoquine (ARCO) phase IV, single center, 2 arms randomized controlled study that evaluated safety, tolerability, efficacy and pharmacokinetics of ARCO compared to dihydroartemisinin piperaquine phosphate (Eurartesim). The study was conducted in Bagamoyo clinical trial unity (BCTU) in Bagamoyo District, about 74 kilometers north of Dares-salaam within the coastal region of Tanzania. Patients with malaria symptoms residing within Bagamoyo district seeking care at the health facilities were informed about the study and those interested were tested for malaria using rapid diagnostic tests (RDT). Parasite positive and verbal consenting patients were transferred to the facility for screening and inclusion in the study. Written informed consent was obtained from each patient prior to any study procedure. In the case of children (under 18 years of age), full written consent was provided either by a parent or legal representative, in addition, for children aged between 12 and 17 years, the child gave written assent. The study was approved by the Tanzania food and drug authority (TFDA), and by the local and regional ethics review boards of Ifakara health institute (IHI) and the national institute for medical research (NIMR) respectively. Patients were hospitalized for 3 days and then discharged and followed up over a period of 42 days.

Drug regimen and blood sampling. Patients randomized to ARCO received a single dose of standard treatment on day 0. Each tablet of ARCO contains 125 mg artemisinin and 50 mg of naphthoquine. The total dose for adults was 1000 mg of artemisinin and 400 mg of naphthoquine (8 tablets) and for children the dose was based on body weight (20 mg artemisinin + 8 mg naphthoquine per kg body weight). The drug was orally administered to each study participant under supervision and incase within 30 minutes of administration a patient vomited the drug; the patient was re-dosed with a full dose. No patients receiving ARCO vomited.

Blood samples (3ml) were collected from each patient to obtain measurements of artemisinin and naphthoquine plasma concentrations. The samples were collected 30 minutes prior to dosing (pre-dosing) and thereafter (post dosing) at 1, 2, 4, 8, 12 and 18 hours for both artemisinin and

naphthoquine, and then on days 4, 7, 14, 21, 28 and 42 for naphthoquine only. Plasma was separated from whole blood aliquot into cry vials and stored at BCTU at -80°C before transfer to Swiss BioQuant, Reinach, Switzerland for analysis.

Analytical method. The quantification of artemisinin and naphthoquine concentrations in plasma was performed by column separation with reverse phase chromatography followed by detection with triple-stage quadrupole MS/MS in the selected reaction monitoring mode. Three independent quality control samples at different concentrations were analyzed within each batch to ensure accuracy and precision during analysis. For artemisinin, the quality controls were performed with the following concentrations 3.0, 50.0 and 375.0 ng/mL, and for naphthoquine at concentration of 0.6, 5.0, and 37.5 ng/mL. The coefficient of variation during artemisinin quantification (n = 16 at each concentration) was 8.5%, 3.5%, and 5.7% at 3.0 ng/ml, 50.0 ng/ml, and 375 ng/ml respectively, and for naphthoquine (n = 12 at each concentration) 6.0%, 5.0%, and 4.7% at 0.6, 5.0, and 37.5 ng/mL, respectively. The lower limit of detection was set to 1 ng/mL, and 0.2 ng/mL for artemisinin and naphthoquine, respectively.

Data analysis and pharmacokinetic modeling. Preparation of data sets for the analysis and calculation of summary statistics on age, weight, sex and other demographics, as well as vital and laboratory parameters was undertaken using Stata (version 13, College Station, Texas, USA).
The population pharmacokinetics of artemisinin and naphthoquine plasma concentration-time data were analyzed using non-linear mixed effects methods with NONMEM (linear mixed-effects modeling, Icon Development Solutions, Ellicott City, MD) version 7.3. The first-order conditional estimation method (FOCE) (33) with interaction was used for estimation of the population parameters.

Models were fitted separately for each drug in the ARCO combination and different disposition models (one-, two- or three-compartment) with first order absorption with either lag times or transit compartments (34) were evaluated. Allometric scaling using body weight (BW) and the median weight of 32 kg was employed by default, with volumes of distribution terms multiplied by $(BW/32)^{1.0}$ and clearance terms by $(BW/32)^{0.75}$ (35). Alternative scaling using fat free mass (FFM) was also investigated (36). The minimum value of the objective function (OFV) and visual predictive checks (VPCs) were used to guide selection of suitable models. The OFV as calculated using NONMEM follows a χ^2 distribution. Interindividual variability terms were introduced after each step of the structural model development and were removed if not supported by the data. The variability terms in each parameter were described using a log-normal distribution as described by equation $\theta_i = \theta \times \exp\left(\eta_{i,\theta}\right)$. Where θ_i is the individual parameter, θ is the median value of parameter in the population and $\eta_{i,\theta}$ is the interindividual variability, which is normally distributed with zero mean and variance ω^2.

The M6 method (37) was used to handle values below the limit of quantification for each patient. For this method, the first value of below limit of quantification is replaced by half the lower limit of quantification and the rest were ignored for the fit but included for the diagnostic plots. A combined additive and proportional error model were used to describe unexplained residual variability.

The relationships between model parameters and the baseline covariates age, parasitemia, hemoglobin, haematocrit, glomerular filtration rate, body mass index, sex and fever were evaluated through inspection of scatter plots (continuous covariates) and box plots (categorical covariates). Post-hoc values of inter-individual variability distributions were evaluated using changes in objective function values obtained from NONMEM. A stepwise forward inclusion algorithm with inclusion criteria of $P < 0.05$ and backward elimination with retaining criteria of $P < 0.001$ was used to test covariate relationships (38).

Various goodness-of-fit plots were used as diagnostic tools (39) to guide model selection. Interval estimates, including those shown in the VPC, were obtained using a non-parametric bootstrap in Perl-speaks-NONMEM (PsN) (40). These were based on 1000 generated resampled datasets, each stratified by age group. Ideally weight could be used for stratification but since weight and age were correlated, we would expect the same distribution as when weight is used. Furthermore, since the patients were enrolled according to their age group, it was more useful to stratify by age group to maintain the original age distribution.

Simulation. Individual parameter estimates were obtained from the final model and used to simulate day 7 concentration and maximum plasma concentration (C_{max}). The steady-state C_{max} was used as measure of safety, and the day 7 concentration as a measure of efficacy. The simulated values were compared across different weight bands, defined according to the manufacturer's recommendation (Kunming pharmaceuticals, Kunming, China). Individual demographic data from malaria patients from Burkina Faso, Ghana, Mozambique and Tanzania (n=833), data from INDEPTH network – INESS study (41), and from a malaria surveillance study (n = 500) from the Bagamoyo research and training center (Ifakara Health Institute) – Tanzania (data not published) were used to define the characteristics of simulated patients. A total of 1393 in silico patients with a minimum weight (16 kg) were thus available for simulation (with 16 kg equal to minimum recommended by manufacture).

The final population PK models were simulated 5000 times using the manufacturers dosing guidelines and the predicted median and ranges for day 7 and C_{max} values for each weight band were collated. A reference patient was defined as weighing 50 kg. The cut-off value for exposure level was based on 5000 simulations of the reference patient. In a previous study (11), 400 mg of naphthoquine (ARCO) was given to malaria patients of average weight 48 kg and they found cure rate of 98%. In this study 400 mg naphthoquine was given to patient with 50 kg body weight. The naphthoquine day 7

concentration has been shown to correlate with overall exposure (area under the plasma concentration-time curve) (14). Different weight bands were evaluated for each available tablet strength for naphthoquine.

For safety, the value of C_{max} from healthy subjects who received 600 mg naphthoquine was used as threshold, corresponding to the maximum dose of naphthoquine delivered without resulting in any safety concerns (42). In each weight band a new dose was recommended if the median day 7 concentration for each weight was less than 80% of median naphthoquine day 7 concentration of the reference patient while maintaining the median C_{max} below the threshold value.

We split a single dose of 400 mg given to reference patient weighing 50 kg (based on manufacturer dosing) to two- and three-days doses to assess the overall exposure between the three regimens (single dose, double doses or triple doses). For two days dosing, 200 mg/day was used while for 3 days the regimen of 133 mg/day was used. Dose interval for the multiple dose was set to be 24 hours. Simulations (5000) for a reference patient weighing 50 kg were performed and for each dosing group plasma concentration-time profile was generated and compared between the three scenarios.

5.4 Results

A total of 29 Tanzanian patients with uncomplicated falciparum malaria were enrolled. Medan age (range, IQR) and weight at baseline was 13.1 (6.0 – 56.0, 8.1 – 21.1) years and 32.0 (20 – 84, 22.0 - 54.1) kg, respectively. Baseline characteristics of the study population are shown in Table 5.1. Overall, the median artemisinin dose was 18.5 [IQR: 16.7 - 18.8] mg/kg, with the lowest dose administered to older patients (median: 16.9, IQR: 15.4 - 18.5 mg/kg). For naphthoquine, overall median dose was 7.4 (IQR: 6.7 - 7.5) mg/kg and patients aged 18 years and above received the lowest dose (median: 6.8, IQR: 6.2 - 7.4 mg/kg) Table 5.1. One subject (Subject 21001-33) received 8 tablets instead of 6 tablets based on body weight (48 kg) was included in the analysis. No subject was reported to vomit the drug.

Pharmacokinetic modeling. All 29 patients had 6 samples collected according to protocol (6 samples per a patient) for artemisinin and none of the 174 samples were below the limit of quantification. The observed artemisinin concentration-time data was best described by 13 transit-compartments in the absorption phase with a mean transit time of 0.81 hours followed by one distribution-compartment. For the distribution phase, an addition of a second compartment improved the model fit in terms of change in objective function value (ΔOFV = -13.6) but was not considered as a final model since there was no obvious improvement in the visual predictive check (Figure 3). Thus, the data supported a one compartment disposition model. Relative bioavailability (F) was allowed to vary between subjects leading to the final structural model shown in Figure 5.1 with parameter estimates given in Table 5.2.

Incorporating body weight as an allometric function on clearance and volume parameters resulted in a significantly better fit compared to the base model for artemisinin (ΔOFV = - 17.9, 1 degree of freedom, p-value < 0.001 and ΔOFV = - 4.6, 1 degree of freedom, p-value = 0.03 respectively) and also inter-individual variability decreased for clearance and volume by 16% and 3.3%, respectively. No other covariate was significant. Inter-individual variability was estimated on clearance (CL/F), volume of distribution (V/F), absorption rate constant (K_a), mean transit time (MTT) and relative bioavailability (F). A visual predictive check for the final model (n = 1000) is depicted in Figure 5.3.

Thirteen samples per patient were required as per protocol, but only 363 venous plasma samples naphthoquine drug concentrations were available, of which five (1.4 %) samples were below limit of quantification (BLOQ). The median sample was 13 per subject with minimum of 9 and maximum of 13 samples. The naphthoquine concentration-time profile was best described by 10 transit-compartment with mean transit time of 1.01 hours in the absorption phase followed by a 3-distribution compartment model. A transit compartment model was superior (Δ OFV = -65.7) to a lag time model (Δ OFV = - 23.8). For the distribution phase, a three-compartment model was significantly better than a two-compartment model (Δ OFV = - 23.0). Inter-individual variability in bioavailability was estimated,

and Figure 5.2 and Table 5.2 depict the final structural model and parameter estimates, with a non-parametric bootstrap used to estimate the precision on the parameters (n=1000).

Adding fat-free mass as an allometric function on the volume of the central and second peripheral compartments of naphthoquine improved the OFV by 5.6 and 17.9, respectively, compared to using body weight. Adding other covariates did not improve the model fit. Inter-individual variability was estimable for CL/F, V/F, K_a, MTT, and F. A visual predictive check indicates the model described the data well (Figure 5.4). Overall predicted median (range) day 7 concentration, C_{max} and time to reach maximum concentration were respectively 13.2 (6.71 - 20.5) mg/L, 44.5 (21.2 – 131) mg/L and 4.6 (2.27 - 13.7) h. Children aged 11-17 years have lower exposure level than other age groups (Table 5.4).

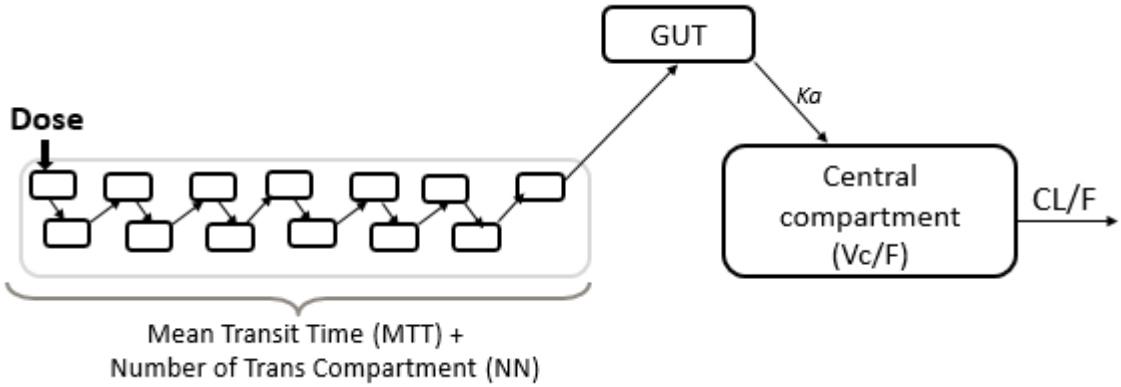

Figure 5.1 Structural presentation of the final model describing population pharmacokinetics of artemisinin in Tanzanian malaria patients. Ka, absorption rate constant; CL, clearance rate; Vc, central volume of distribution; F, relative oral bioavailability

Table 5.2 Parameter estimates of the final model describing population pharmacokinetics for artemisinin and naphthoquine in patients with uncomplicated *P.falciparum* malaria in Tanzania

	Original data	Bootstrap: 95 % CI[c]
Parameter[a]	Estimates[b]	Estimates
Artemisinin		
Ka [1/h]	1.17	1.11 - 1.22
MTT [h]	0.813	0.602 - 1.2
NN	13	2.55 - 27.4
F	1 Fixed	-
CL [liters/h]	44.2	38.2 - 52.6
Vc [liters]	222	187 - 268
Additive error [ng/mL]	0.05	0.05 - 18.5
Proportional error (%)	29.4	24.7 - 38.5
Interindividual variability (% CV)		
Ka [1/h]	53.1	53.0 - 130
MTT [h]	50.3	27.1 - 109
CL [L/h]	7.3	7.0 - 22.0
Vc [L]	19	19.0 - 24.0
F	41	23.3 - 47.8
Naphthoquine		
Ka [1/h]	0.638	0.32 - 1.36
MTT [h]	1.01	0.79 - 1.68
NN	10	3 - 18
F	1 Fixed	
CL [L /h]	18.3	9.4 - 28.0
Vc [L]	3330	2164 - 4481
Q_1 [L/h]	18.1	11.0 - 29.8
V_{p1} [L]	23800	5143 - 49738
Q2 [L/h]	360	269 - 492
Vp2 [L]	6520	4778 - 7807
Additive error [ng/mL]	0.364	0.08 - 0.86
Proportional error (%)	24.2	0.19 - 0.27
Interindividual variability (% CV)		
Ka [1/h]	68.1	0 - 98
MTT [h]	74.6	47.9 - 106
CL [L/h]	21.2	0 - 34.6
Vc [L]	32.7	0 - 51.0
Q1 [L/h]	46.3	0 - 92.7
F	32.2	24.5 - 40.0

[a] K_a, absorption rate constant; MTT, mean transit time; NN, number of transit compartment; F, relative bioavailability; CL/F, oral clearance; Vc/F, central volume of distribution; Q/F, inter-compartmental clearance; Vp/F, peripheral volume of distribution.

[b] Computed population mean parameter estimates from NONMEM.

[c] Computed from nonparametric bootstrap method of the final model (n = 500).

Interindividual variability was assumed as log-normally distributed and it is reported as approximate %CV calculated as $(\text{estimate})^{½} \times 100$.

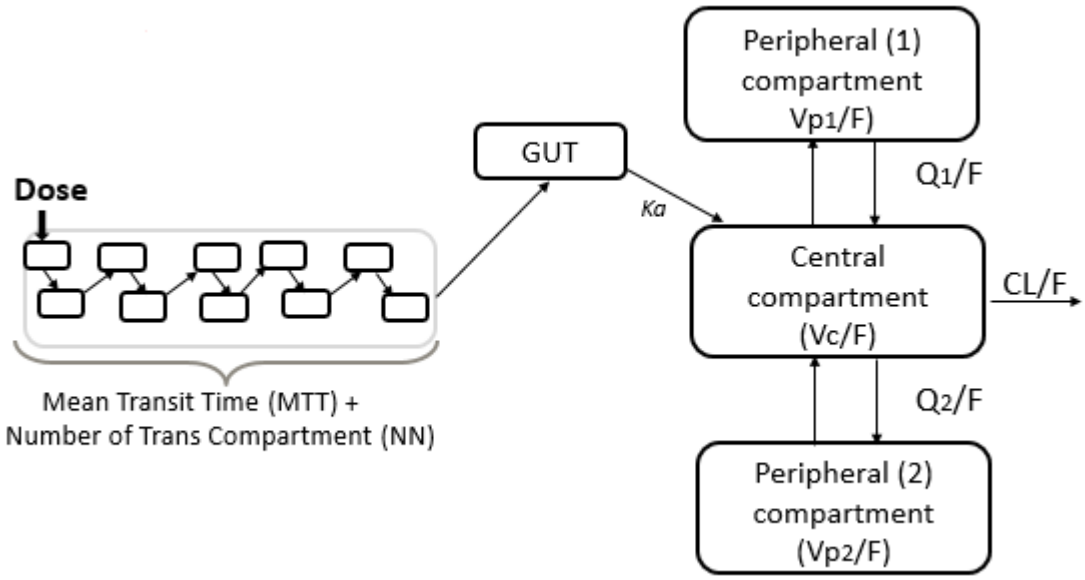

Figure 5.2 Structural presentation of the final model describing population pharmacokinetics of naphthoquine in Tanzanian malaria patients. Ka, absorption rate constant; CL, clearance rate; Vc, central volume of distribution; Q, intercompartment clearance; Vp, apparent volume of distribution of the peripheral compartment; F, relative oral bioavailability.

Simulation. The day 7 concentration of naphthoquine for reference, 50 kg, patient was predicted to be 14.0 (IQR = 11.0 – 17.7) ng/mL from 5000 simulations. Figure 5a summarizes the results for different weight ranges using the current recommended dose and indicated that the day 7 concentration of naphthoquine for children who weigh 29 – 32 kg, and patients 46 – 49 kg and > 63 kg are have on average 25% lower exposure than a 50 kg patient.

The threshold for efficacy, defined as 80% of the median naphthoquine day 7 concentration for the reference patient taking a single dose of 400 mg, was found to be 11.2 ng/mL, while for toxicity, the maximum concentration of 60 ng/mL was used. A new dosing regimen for different weight bands using these optimized thresholds is summarized in Table 5.3, together with the current recommended doses. The optimized dosing regimen includes higher doses per kg for younger children to achieve comparable exposure across weight bands without any risk of toxicity Figure 5.5d.

Day 7 concentration for a patient receiving single dose of 400 mg was 13.8 (IQR: 10.9 – 17.5) ng/mL, C_{max} of 53.7 (40.1-72.1) ng/mL. When the dose is split for 2 days, the day 7 concentration and C_{max} were 14.3 (IQR: 11.3 – 18.1) and 38.3 (29.5-49.9) ng/mL, respectively. For three-day regimens, the day 7 concentration was 14.8 (IQR: 11.7 – 18.8) ng/mL and C_{max} was 32.2 (25.0-41.3) ng/mL.

Table 5.3 Current dose regimen and optimized dose regimen based on simulations for naphthoquine

Currently dose regimen			Simulated based dose regimen		
Body weight (Kg)	Number of tablets	NPQ (mg)	Body weight (Kg)	Number of tablets	NPQ (mg)
≥ 16 to < 21	3 x 50	150	≥ 16 to < 20	3 x 50	150
≥ 21 to < 33	4 x 50	200	≥ 20 to < 25	4 x 50	200
≥ 33 to < 50	6 x 50	300	≥ 25 to < 36	5 x 50	250
≥ 50	8 x 50	400	≥ 36 to < 43	6 x 50	300
			≥ 43 to < 60	8 x 50	400
			≥ 60	10 x 50	500

5.5 Discussion

There is increasing interest in using artemisinin and naphthoquine (ARCO) as an alternative ACT in malaria endemic Sub-Saharan African countries, including Tanzania. Several other studies have examined pharmacokinetics properties of artemisinin in this population when given alone or in combination with other partner drugs as a single dose therapy. But despite use in routine clinical practice and several PK studies conducted in Papua New Guinea (PNG), this is the first time a population PK analysis of naphthoquine given in combination with artemisinin has been reported in a Tanzanian population. Characterization of the PK of naphthoquine is essential to ensure evidence based optimized dosing and to ensure appropriate regimes from single to multiple dosing are chosen.

A one-compartmental model was found to best describe artemisinin pharmacokinetics, which differs structurally from several previously reported studies. This is most likely due to the single artemisinin dose and limited PK data in follow-up of this study. One or two compartmental, as well as semi-mechanistic and semi-physiological with auto-induction and first-pass hepatic extraction models have been found in healthy and malaria patients (43–48). Artemisinin has been reported to induce its own metabolism when repeated doses are given (49) which could explain the better fit of auto-induction models in studies where patients were treated with a multiple dose of artemisinin. In the present study, where only a single dose was given, the auto-induction model could not be tested. Moreover, in this relatively small study, no covariate relationship was found to improve population pharmacokinetic parameters.

A three-compartmental disposition model, and multi-compartment transit model for absorption, best described the population pharmacokinetics of naphthoquine. This finding is in line with previous findings from an artemisinin - naphthoquine study in PNG (14). Similar findings have been reported for another quinolone antimalarials, for example chloroquine (51–53) and piperaquine (32, 54). In PNG, patients with fever were associated with a 32% decrease in relative oral bioavailability. Furthermore, in this study a one g/dL increase in patient haemoglobin level was associated with a 16%

increase in volume of central compartment (14). In the present analysis, no appropriate parameter-covariate relationship was found. In general, pharmacokinetic parameters (oral clearance and volume of distribution) were more precise, but lower in our study (Table 5.2) than in the PNG study (14), even after adjusting for body weight allometric scaling. This might be attributed to the lower bioavailability due to fever in the PNG study (14).

The PK models described in this paper provide further evidence to explore alternative dosage regimes. Naphthoquine is predicted to achieve a maximum concentration in 4.6 h of 44.5 (range: 21.2-131) mg/L over all patients. In children less than 11 years of age (median: 20 kg), this maximum was lower by 26% compared to adults of more than 18 years of age (median: 55 kg). The mean elimination time of naphthoquine was found to be 123.1 [59.8 – 268 h] in malaria patients, in agreement with previous studies of healthy volunteer in China (156 to 299 h) (42) where elimination was slower compared to pediatric malaria patients in PNG (524 h) (14).

The final PK model for naphthoquine demonstrated adequate predictive performance and could be used to evaluate the current dosing in children receiving a single dose of naphthoquine (when given in combination with artemisinin). The simulations suggest that the manufacturer's current dose recommendation might be too broad, resulting in slight under dosing of children at the upper end of each weight group (Figure 5.5). Those weighing 29 - 32 kg will require higher doses than currently recommended in order to achieve optimal plasma concentrations (based on the 50 kg adult). Additional doses are also required for patients weighing 46– 49 kg and > 63 kg. Low exposure level in patients may also increase spread of resistance, in particular in young children whose immunity to blood stage malaria parasites is low (55).

The preliminary revised dose scheme for naphthoquine investigated here indicates a higher dose in young children (Table 5.3), this adjustment will give similar plasma naphthoquine exposure across all weight groups without risk of toxicity Figure 5.5d, using tablet strengths in line with currently manufactured tablets. However, since naphthoquine is in fixed combination with artemisinin in ARCO, further analysis with more artemisinin data and optimization should be undertaken with the partner drug artemisinin, on both exposure and safety.

The proposed and optimized single dose regimen (Table 5.3 and Figure 5.5) was constructed to ensure that the median day 7 plasma naphthoquine concentration was not less than 80% of the median of the typical patient (patient weight 50 kg). Given the fixed formulation of ARCO, this results in a median dose of artemisinin 19.5 (range 15.6–25) mg/kg, which is also higher than the recommended 17.2 mg/kg. At the current dosing, ARCO is well tolerated (11, 22, 42, 56, 57). The increased dose in the preliminary optimization results in a maximum dose of about 13% higher of both

artemisinin and naphthoquine and would not be expected to reduce tolerability. In one study in PNG (12), artemisinin was administered at higher doses (23.8 mg/kg) together with naphthoquine (9.5 mg/kg) to 5 – 12 years children with uncomplicated malaria. Despite the dose being well tolerated with no serious adverse events there was an observed QTc prolongation in artemisinin - naphthoquine treated children 4 hours after the third dose in PNG when given as three days regimen (58). Therefore, the safety and tolerability of any increases in doses, such as proposed here, would need to be thoroughly evaluated.

For the dose investigation in this study in over 5 years of age, children of body weights 16 – 19 kg were included based by parameterizing a synthetic population based on data from INDEPTH. This is because the clinical trial did not include patients of this weight range (observed weights 20 kg to 84 kg). Thus, the simulation results should not be further extrapolated below this weight, and dose investigations for weights under 16kg should be informed with data from other trials. Despite this, the model simulations did not suggest any need for dose adjustment when predicting for slightly lower weight ranges than those in trial.

ARCO has been recommended and formulated to be used as a single dose to address issues of adherence. However, there is evidence that multi-doses of ARCO will result in higher efficacy, for example in a study where patients received ARCO twice a day resulted in higher cure rate than those receiving single dose (13). Furthermore, given growing concerns surrounding artemisinin resistance there is strong argument against single doses of artemisinin (18, 19). Our simulations show that moving from a single fixed dose of 400 mg (NPQ) to a three-day dosing regimen (133 mg/day) or a two-day schedule (200 mg/day) is favorable for safety (maximum concentration) and will increase exposure level (day 7 concentration). Day 7 concentration have been shown to correlate with area under the curve and also cure rate for naphthoquine (14). The modelling results in the manuscript thus provide further pharmacokinetic evidence of higher efficacy for multi-day dosing, as reported in an earlier clinical study (Meremikwu et al. 2012) and in Batty et al. 2012 (14) as compared to those received single dose. Taking this modelling and clinical evidence together, ARCO treatment regimens should potentially adopt the currently recommended three days dosing regimen for ACTs by WHO (59). Because the number of patients recrudesce cases were low in our clinical study, this analysis could not assess the pharmacodynamics (PD) aspect of the naphthoquine, and therefore more study is required to assess the safety and efficacy of the proposed dosing regimen.

Our study has a number of limitations primarily concerned with using data from a trial with a small number of patients treated with a single dose of ARCO alone, as well as limited PD data to parameterize exposure response relationships. Further clinical studies and pooled analysis of all PK studies in multiple populations of naphthoquine alone, or in combination, is warranted. In particular,

the inclusion of PK studies in a pooled analysis with lower dosing that resulted in sub-curative treatment or recrudescence at follow-up, thus informing appropriate modelling of the PD of naphthoquine. Such models will allow further in-silico investigations of optimal dosing schedules to address resistance concerns for a single dose of artemisinin in the current recommendation of ARCO.

In conclusion, this study described the population pharmacokinetic properties of artemisinin and naphthoquine in patients with uncomplicated malaria in Tanzania. Population simulation suggest that young children receive lower naphthoquine exposure based on the current dose recommendation, and hence warranting potential dose increases such as the weight-adjusted and convenient dosing proposed. Furthermore, our results provide evidence that multiple doses of ARCO may be beneficial, and more analysis is necessary.

5.6 Acknowledgements

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We declare no conflict of interest

Ali M. Ali received funding from Basel Stadt as support for his PhD.

5.7 Appendix

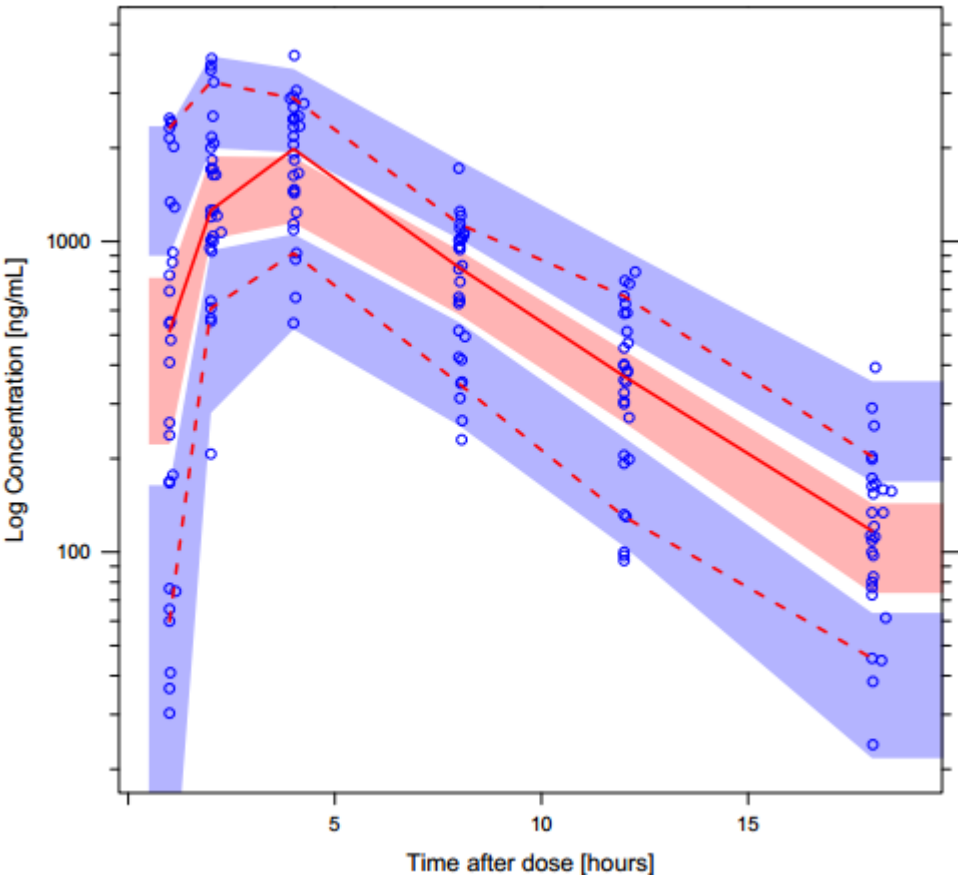


Figure 5.3 Visual predictive check of the final model describing artemisinin population pharmacokinetics in uncomplicated malaria patients in Tanzania. Open circles are the observed data points; solid and dashed lines are the 50th, 5th, and 95th percentiles of the observed data; shaded areas are the simulated (n=1000) 95% confidence interval for the same percentile.

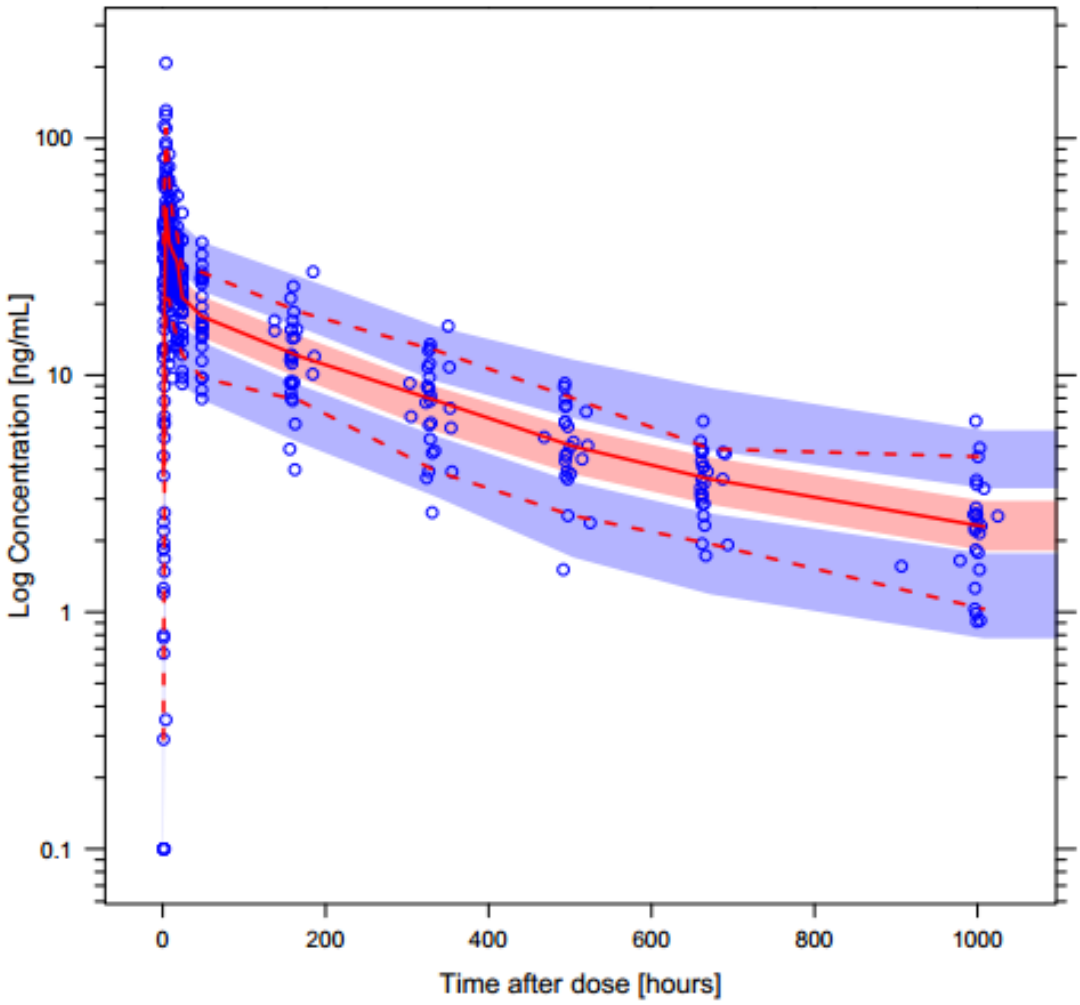
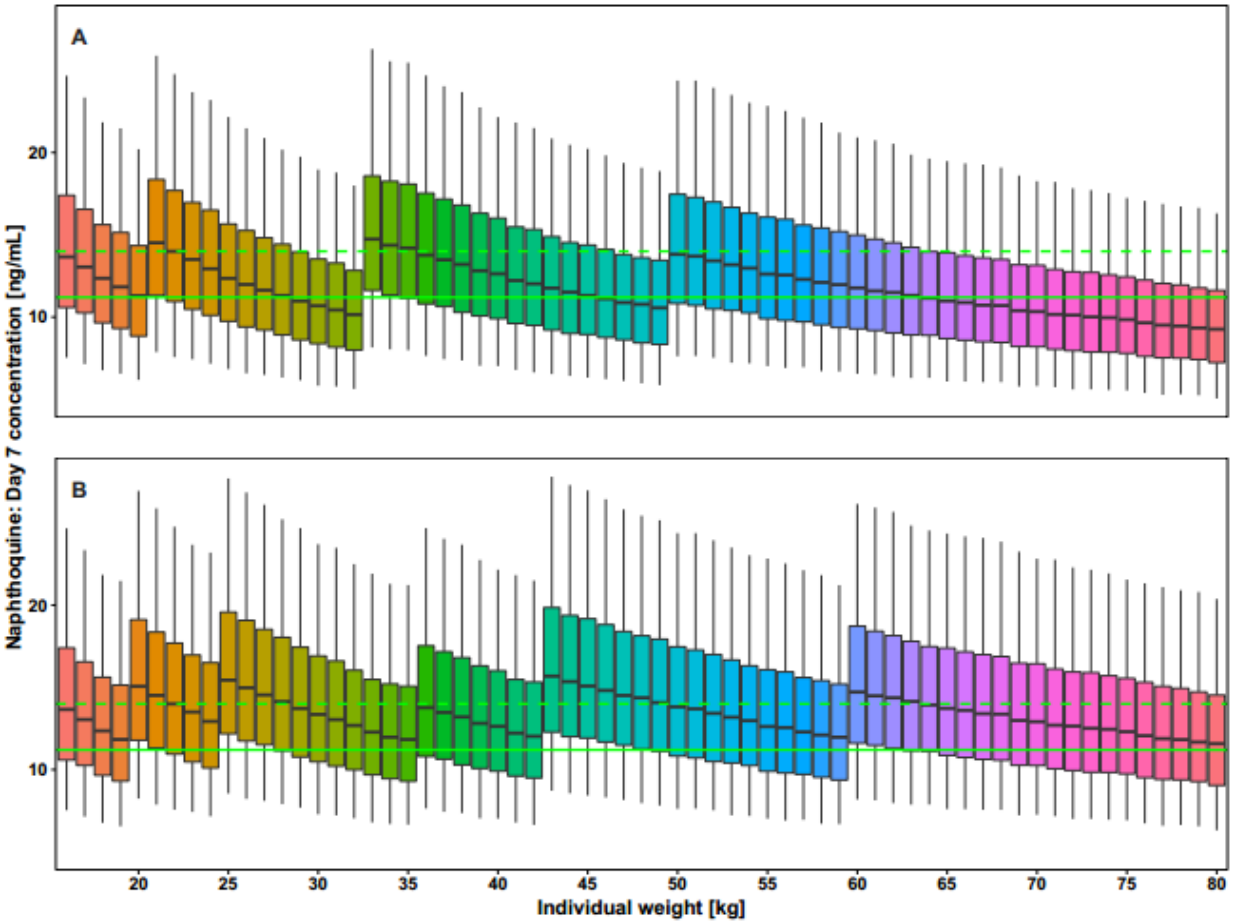


Figure 5.4 Visual predictive check of the final model describing naphthoquine population pharmacokinetics in uncomplicated malaria patients in Tanzania. Open circles are the observed data points; solid and dashed lines are the 50th, 5th, and 95th percentiles of the observed data; shaded areas are the simulated (n=1000) 95% confidence interval for the same percentile.


A
B
20
10
20
10
Naphthoquine: Day 7 concentration [ng/mL]
20
25
30
35
40
45
50
55
60
65
70
75
80
Individual weight [kg]

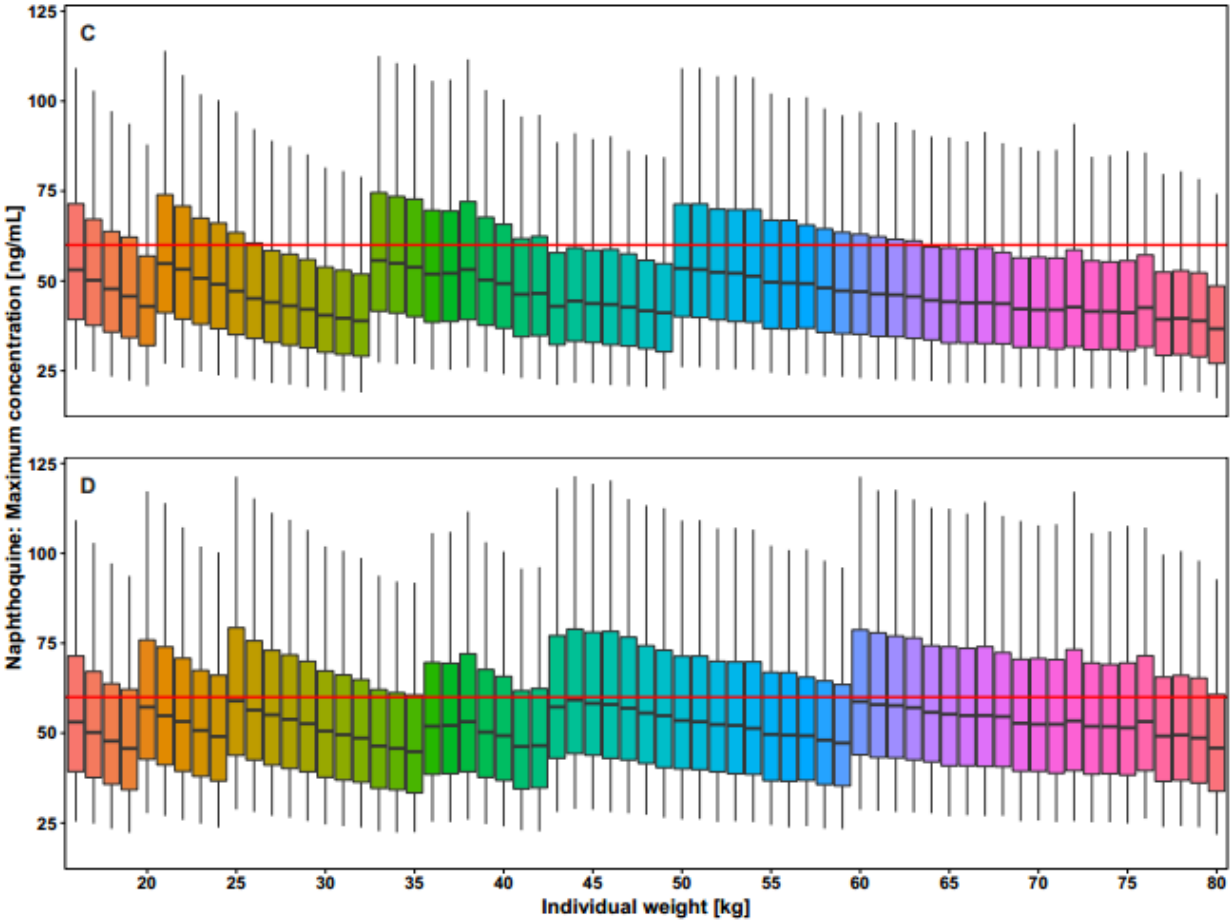


Figure 5.5 Simulation results of current recommended and optimized dose regimens for naphthoquine. Panel **A**: day 7 plasma naphthoquine concentration and **C:** maximum concentration of naphthoquine based on the current dose. **B**: day 7 concentration and **D:** Cmax of naphthoquine for the optimized dose regimen. Green dashed and solid lines in **A** and **B** are the median and 80% value of median of the simulated day 7 plasma naphthoquine concentration of the typical patient respectively and the red line in **C** and **D** represents the Cmax threshold (60 ng/mL). Simulations for each weight are presented as boxplot for the median and 25th and 75th percentile, with whiskers for the 5th and 95th percentiles.

Table 5.1 Baseline characteristics of the study population[a]

	All	6 - 10 years	11 - 17 years	18 + years
Total number of patients	29	12	6	11
ART median (IQR) total dose (mg/kg)	18.5 (16.7 - 18.8)	18.8 (18.6 - 18.8)	18.7 (17.4 - 20.8)	16.9 (15.4 - 18.5)
NPQ median (IQR) total dose (mg/kg)	7.4 (6.7 - 7.5)	7.5 (7.4 - 7.5)	7.5 (7.0 - 8.3)	6.8 (6.2 - 7.4)
ART median number of samples/patient	6	6	6	6
NPQ Median number of samples/patient	13	13	13	13
Male	15 (51.7 %)	3 (25.9 %)	4 (66.7 %)	8 (72.7 %)
Female	14 (48.3 %)	9 (75.0 %)	2 (33.3 %)	3 (27.3 %)
Median (IQR) age (yr)	13.1 (8.1 - 21.1)	7.1 (6.7 - 9.0)	13.5 (12.9 - 14.1)	26.6 (21.0 - 44.9)
Enrolment demographic, vital and laboratory parameters				
Median weight (IQR) (kg)	32.0 (22.0 - 54.1)	20.0 (20.0 - 24.5)	37.5 (26.0 - 48.0)	55.0 (51.0 - 64.0)
Median (IQR) height (cm)	145.0 (122.0 - 162.0)	120.5 (117.0 - 126.5)	149.0 (136.0 - 162.0)	162.0 (155.0 - 172.0)
Median (IQR) body mass index (kg/m^2)	16.4 (14.6 - 20.4)	14.7 (14.1 - 15.5)	15.8 (14.1 - 20.5)	20.4 (19.0 - 25.3)
GM (95 % CI) parasitemia (parasites/µL)	652.7 (427.8 - 995.8)	951.9 (430.8 - 2103.4)	697.6 (236.5 - 2057.6)	417.0 (227.3 - 764.8)
Median (IQR) haemoglobin (g/dL)	12.2 (11.3 - 13.7)	11.8 (11.2 - 12.3)	12.0 (11.1 - 12.7)	13.7 (11.8 - 14.3)
Median (IQR) haematocrit (%)	36.4 (33.5 - 39.8)	35.5 (33.5 - 37.2)	36.0 (32.4 - 37.7)	39.9 (34.6 - 41.8)

[a]Percentages can be more or less than 100% due to rounding error; ART = Artemisinin, NPQ = Naphthoquine; GM = Geometric mean; IQR = Interquartile range

Table 5.4 Secondary parameter of the final population pharmacokinetic model of NQP in Tanzanian malaria patients

Parameter	All	6 - 10 years	11 - 17 years	18+ years
Day 7 concentration (ng/mL)	13.2 [6.71 - 20.5]	13.6 [7.32 - 19.1]	11.3 [7.58 - 20.5]	14.0 [6.71 - 17.9]
Cmax (ng/mL)	44.5 [21.2 - 131]	48.5 [23.9 - 79.2]	37.8 [24.5 - 131]	38.4 [21.2 - 95.6]
Tmax (hours)	4.6 [2.27 - 13.7]	4.35 [2.75 - 7.32]	4.39 [3.28- 6.72]	5.19 [2.28 - 13.7]
t1/2 (hours)	123.1 [59.8 - 268]	123 [86.6 - 175]	125 [59.8 - 176]	123 [96.9 - 268]
AUC day0-42	7283 [3760 - 11096]	7655 [4110 - 10623]	6247 [4383 - 11096]	7283 [3760 - 9798]

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Chapter six

This chapter provides a discussion of all findings in this study and draws a meaningful conclusion. Moreover, it provides suggestions for public health improvement, implication for future research, implication for new malaria drugs in development, and implication for policy and practice.

6. Overall discussion and conclusions

Despite recent huge declines in malaria burden, malaria still poses an international threat (1). The large scale adoption of artemisinin combination therapy (ACTs) as first line treatment for malaria, in addition to vector control, has contributed to these reductions. ACTs have to a large extent also enabled many settings to overcome consequences of drug resistance as a result of monotherapy. However, there is presently indication of increasing resistance to some ACTs and the threat of wide-spread drug resistance is looming (2–4) The consequences of drug resistance, as well as failed treatments, is most severe in children, especially in malaria endemics settings where children generally receive more treatment compared to adults who may have higher immunity (1).

Children have been exposed to sub optimal doses when receiving antimalarial treatment, as reported in a number of studies (5–10). The current practice of dose finding in children has contributed to treatment failure in children (11) because, while in adults dose finding is based on a number of clinical trials determining efficacy and safety of the dose regimen, in children optimal doses are mainly determined by extrapolation of adult doses using body weight, age or body surface area (12–16). Given clinical trials of new drugs necessarily do not include children, the appropriate choice of drug dosing in children becomes particularly challenging (13).

Motivated to improve treatment protocols for vulnerable populations, in particular children, the general aim of this thesis was therefore to increase the scientific understanding in regards dose optimazation in children for antimalarials. This thesis provided the model based proof of the suboptimal dosing of two antimalarials by using data from 8 clinical studies and employing population pharmacokinetic (pop-PK) modelling and simulation. The modelling allowed understanding of the absorption and elimination of antimalarials, the factors influencing these dynamics and comparison of exposure in different populations to ensure adequate curative treatment. These models were used to explore optimal doses or regimens with special focus on young children to assess if children are receiving the right dose based on the current dose recommendation. Two anti-malaria drugs were used; amodiaquine (Chapters 3 via a pooled analysis of several studies and Chapter 4 an analysis of one study) and naphthoquine (Chapter 5, analysis of one clinical study). These drugs have longer elimination half-lives (17–19) compared to their partner drug in their respective combination therapies (20–23) and hence are responsible for the majority of the anti-malarial activity.

Detailed discussions on the main findings for each drug, model and model investigation can be found in each of the respective chapters. In this section, the main findings are summarized and critically discussed. The implications of the findings for drug development and future research, as well as the implications for policy and practice, are further discussed. Finally, I conclude with a summary of main conclusions.

6.1 Main findings

6.1.1 Amodiaquine kinetics (Chapter 3 & 4)

The pooled pop-PK modelling of amodiaquine in chapter 3 was developed using data from six different studies (5 cohorts) undertaken in five countries (pooled analysis). Venous plasma concentration was used for amodiaquine quantification. A separate analysis on data not included in the pooled analysis was completed for the pop-PK of amodiaquine (Chapter 4). This was owing to data availability and timing of the study, as well as the fact this study used different biologic fluid (capillary whole blood) from patients.

6.1.2 Venous plasma concentration (Chapter 3)

The studies were conducted predominantly in sub-Saharan African countries, and with one study in Asia, covering a wide range of patient ages, different malaria species infections, and transmission (24–29). A total of 261 patients were used in the analysis. Amodiaquine was administered either alone or with artesunate (AS) as loose or fixed-dose combination therapy. Treatment was given once daily for 3 days at a target dose of 10 mg/kg of body weight.

In this pooled analysis, a two-compartment model best described amodiaquine disposition while desethylamodiaquine (DEAQ) was described best with a three-compartment disposition model. The important covariates were body weight on clearance and volume of distribution, age maturation effect on clearance of amodiaquine and desethylamodiaquine, and effect of occasion on bioavailability of amodiaquine.

Two compartmental models have been found to best describe amodiaquine kinetics in several studies, for example in a study of Asian pregnant women (25). And also in agreement with other quinolone compounds that are generally 2 compartments (30–35). In contrast, few studies have reported use of one compartment model for the disposition of amodiaquine (24, 27).

6.1.3 Capillary whole blood concentration (Chapter 4)

For the pop-PK modelling of amodiaquine in chapter 4, data from both children and adults with malaria from Navrongo in Ghana, who gave capillary whole blood samples was used. Fixed dose artesunate-amodiaquine combination therapy was administered once daily for 3 days at a target dose of 10 mg/kg of body weight. Two compartment model was found to describe well both amodiaquine and desethylamodiaquine. Body weight as allometric scaling on clearance and volume of distribution was the most important covariate found. Other important covariates include, age maturation effect on clearance of amodiaquine and desethylamodiaquine, effect of hemoglobin (HB) on bioavailability of amodiaquine and parasitemia was found to affect the clearance of amodiaquine.

6.1.4 Comparison of amodiaquine kinetics in this thesis

Pop-PK modelling should always use the fewest number of compartments necessary to describe the concentration-time data adequately. There are several factors that determine the number of compartments of a certain drug. These factors include (i) the route of drug administration, (ii) the rate of drug absorption, (iii) the total time for blood sampling, (iv) the number of samples taken within the collection period, and (v) the assay sensitivity (36). The use of either one-, two-, or three compartment is can be driven by design and the purpose of the study. Improved pharmacokinetic study design, including more-frequent sampling of longer duration, as well as lower limits of quantification for the analytical techniques, may explain the use of more-complex elimination kinetics.

A three compartment was found to best describe desethylamodiaquine kinetics (Chapter 3) which is different to the model with the capillary whole blood (Chapter 4), where a two-compartment model was found. The pooled analysis was first developed using data from Asian pregnant women (25), which has relatively more samples and longer follow-up period than the other studies in the pooled analysis. Combining this data with other datasets with fewer samples did not significantly alter the structural model even though 62.1% patients had only sparse sampling (range: 1-3 samples per subject) (Chapter 3). In agreement with our results, a three compartmental model was found to best described desethylamodiaquine disposition in healthy adult volunteers who received a single oral dose of 10 mg/kg amodiaquine (37). In comparison to Chapter 3, the shorter follow-up and higher proportion of data below limit of quantification in Chapter 4 (capillary whole blood) most likely explains why a three-compartment model was no better than two compartments.

In a study of Asian pregnant women, desethylamodiaquine was still detected in plasma at day 42 and the LLOQ was set at 2 ng/mL (25). For the study with capillary whole blood (Chapter 4), patients were followed up to day 28. Higher concentration (median [IQR]: 53 [26 – 106] ng/mL) of desethylamodiaquine was detected at day 28 and the LLOQ was set at 0.781 ng/mL. Higher number of BLOQ data at the terminal elimination phase could also influence the use of fewer compartments. Several other studies also found a two compartmental model for desethylamodiaquine concentration-time profile (24, 27). One of the these studies collected two samples on day 3 and day 7 per patient (24).

6.1.5 Amodiaquine covariates (Chapter 3 & 4)

Venous plasma concentration (Chapter 3)

Clearance was found to mature with post menstrual age with children at birth estimated to have levels of clearance 27% and 24% of adult levels for amodiaquine and desethylamodiaquine, respectively. In addition, children were estimated to reach levels of clearance of 50% of the weight-adjusted adult values at 3 and 4 months after birth for amodiaquine and desethylamodiaquine, respectively. But only

reaching 95% of the adult value at around 2 years of age and 100% values at about 3 years of age. This is consistent with the maturation of metabolizing enzyme which is expected to reach adult levels by 2-3 years (38). However, we note that the value of clearance for amodiaquine was heavily influenced by our prior values, likely due to (i) lack of data availability for children less than 1 year of age and since the maturation process begins before birth our model relied on assumptions (39), and (ii) a high proportion (55.3%) of the data were below the limit of quantification for amodiaquine.

Developmental changes during childhood can affect the PK processes of the drug from absorption to elimination. Inclusion of age maturation effect on clearance, in addition to allometric scaling using body weight, suggests that allometric scaling alone cannot predict clearance in young children from adults sufficiently (13, 14, 40). While body weight predicts size, age predicts maturation of body function during both intrauterine and extra-uterine life (41). Clearance of a drug is either by renal excretion or hepatic biotransformation to a metabolite. Renal function in children at birth are estimated to be approximately 25-30% of adult value increases to 50-75% by 6 months and full maturation is expected to occur at the age 2-3 years (42, 43). This is in line with our conclusions in this thesis for amodiaquine which is renally eliminated drug (44, 45). Both size, maturation, and renal function affect the pharmacokinetics of renally eliminated drugs (39, 41, 46). This also agrees with the fact that glomerular filtration rates (GFR), a measure of renal function matures with PMA (46). Additionally, CYP-dependent metabolism is about 50% to 70% of adult's levels when the child is born (38).

Amodiaquine bioavailability was found to increase with the time (day) after drug administration. The lower bioavailability on the first day could partly be explained by this disease effect as we expect rapid resolution of disease in the first 24 hours where by malaria parasites have been cleared by at least 35% when patients are treated with ACT (47, 48). On the other side, it could also indicate the need to administer higher doses to malarial patients during the acute phase of the illness. Winstanley et al 1990 (49) reported higher AUC for amodiaquine in healthy volunteers than in malaria patients which could explain the effect of disease on bioavailability. Lower bioavailability in the first day of treatment have been also reported to other antimalarial of similar family with amodiaquine (50). The low bioavailability in day one could also explain the effect of first-pass metabolism which has been shown to affect the oral bioavailability of drug (51, 52).

Pregnancy effect was not significant in our analysis, this may be because of lower number of pregnant patients included in the analysis. Only one study so far reported the effect of pregnancy on the pharmacokinetic parameter of amodiaquine. This was conducted in 27 Asian pregnant women and 19 the same women who came after delivery. In this study pregnancy was found to affect the absorption parameter (25). Pregnancy has been shown to affect elimination clearance and relative

bioavailability (53), apparent volume of distribution (54) of piperaquine and relative bioavailability of dihydroartemisinin (53).

Although disease effect was not significant in our analysis, an indication of presence of disease effect was observed. Nicholas White et al 1987 (55) studied the pharmacokinetic of intravenous amodiaquine in both healthy subjects and malaria patients, he found that clearance of intravenous amodiaquine in malaria patients was lower (5.5 L kg^{-1} h^{-1}) compared to health volunteer (13.0 L kg^{-1} h^{-1}). Lower area under the plasma concentration-time curve was observed in the malaria patients received amodiaquine oral dose compared to healthy volunteers (49). Which reflect higher bioavailability or lower clearance in malaria patients as compared to health subjects. A negative correlation between fever and volume of distribution of mefloquine have been reported previously (56). Relative bioavailability of piperaquine for both pregnant and non-pregnant malaria patients have shown to increase over time (53). Bioavailability of piperaquine was also observed to increase by 22% in day 2 and by 51% in day 3 compared to day 1 (50). These indicate the effect of the disease on the pharmacokinetic parameters. Disease is more likely in young children in endemic settings due to their lower immunity to the parasite compared to adults, and the inclusion of maturation effect on clearance in the model could have confounding the disease effect. The study involving both malaria infection and healthy subjects is needed to quantify this effect.

In our analysis, no association was found between haemoglobin and pop-PK parameters of amodiaquine. HB was strongly correlated with age, where younger patient tended to have lower HB level. The effect of HB on amodiaquine clearance could be explained by the effect of age on clearance via maturation. In addition, severe anaemia is a common presenting feature for young children with severe malaria. Association between HB and dihydroartemisinin clearance was found in African children with severe malaria receiving intramuscular artesunate (8). This study involved severe cases of malaria where children had lower haemoglobin (median 7.1) compared to patients with uncomplicated malaria used in our analysis (median = 11.4). Effect of drug formulation (whether amodiaquine was administered alone, or with artesunate, either as loose formulations or a fixed dose combination tablet) and HB on bioavailability and HB on amodiaquine clearance was not significant (Chapter 3).

Other covariates that were previously found to be significantly affecting pharmacokinetic parameters of amodiaquine includes age on clearance (25), in a study involving adult Asian pregnant women. In our analysis age effects however were included via a maturation effect.

Capillary whole blood concentration (Chapter 4)

Allometric scaling based on body weight alone cannot predict elimination clearance when involving children less than one year of age. This was explained by the highly significant effect of age maturation

effect on weight adjusted clearance of both amodiaquine and desethylamodiaquine. Age maturation effect found in this analysis confirmed the previous findings from the pooled analysis (Chapter 3). In the pooled analysis, no data were available for children less than one year of age. Including data for children less than one year in Chapter 4 gave the power to detect age maturation effects on amodiaquine clearance. The final parameter estimates for the maturation function was similar to those in pooled analysis (Chapter 3) indicating that amodiaquine clearance depends on maturation of enzymes and can be accounted for with age maturation functions. However, only 4.6% (11/242) patients less than one year were available in this study, therefore further studies with more infants are necessary to robustly estimate the parameters of the maturation function.

Malaria infection is associated with decreasing haemoglobin level especially in young children (57, 58). Higher bioavailability in patients with lower haemoglobin level was found in Chapter 4. Positive correlation was found between age and HB, meaning that young children have higher bioavailability than older children and adults. The effect of HB was not supported by the data in the pooled analysis of Chapter 3. This could be explained by the inclusion of age maturation effects in the pooled analysis where age and HB were also correlated. Including both age maturation effect and HB effect in capillary whole blood analysis could have confounding the effect of each other. However, attempts to remove HB from the model was not supported by the data. We note that effect of HB on the central volume of distribution was previously found for chloroquine, (59) an anti-malaria of the same family with amodiaquine (19, 60).

Plasmodium falciparum undergo repeated rounds of asexual multiplication in red blood cells (RBC). This causes degradation of the RBC of the malaria infected person and the higher the parasitemia can be associated with the reduction in liver blood flow (19). Lower clearance we found with each log-increase in baseline parasitemia suggesting this reduction of liver blood flow in malaria patients.

6.1.6 Naphthoquine kinetics (Chapter 5)

Pop-PK analysis of naphthoquine was applied to data from one clinical trial involving 29 patients aged 6 to 56 years in a study in Tanzania. The concentration-time profile of naphthoquine was well described by a three compartmental model. Only one previous study has reported pop-PK of naphthoquine, and this study involved paediatric malaria patients in Papua New Guinea. A three compartmental model was also found to best describe the concentration time curve for naphthoquine. Concentration-time curve of several drugs of quinolone family was fit by two compartmental model (30–35). More frequent sampling (range: 9 – 13 sample per subject) in our study with longer duration (up to 42 days) may explain the selection of more complex elimination

kinetics. Apart from the effect of body weight on clearance and volume of distribution, no other covariates were found to affect the PK parameters of naphthoquine.

6.1.7 Naphthoquine covariates (Chapter 5)

Body weight which was included as allometric scaling on clearance and volume of distribution was found to be significant in our model of Naphthoquine (Chapter 5). This could be explained by the non-linearity of physiological process as function of weight and wider range of weight in the data set (20 – 84 kg). Non-linear relationships between clearance and body weight have been proposed by Hoford et al. 1996 (61) and have traditionally been used to scale clearance and volume of distribution. Hoford et al. 1996 (61) suggested that, weight be included first to explain differences between individuals and then only later assessed for influences on other factors and thus independent of size.

No other covariates were found to affect pharmacokinetic parameters of naphthoquine in our study. In a study of pediatric patients, fever was associated with 32% decrease in relative bioavailability, as well as the apparent volume of distribution increasing by 16% for each unit increase in baseline HB level (62). In this study all patients (total of 46) were children aged 5 – 12 years, while in our analysis only 12 (41%) were children between the ages of 6-10 years. Very young children normally have higher malaria fevers compared to older children, this is related to high burden of malaria parasite due to their lower immunity to the disease (63) and supports that fever influence kinetics. Food was not controlled in our study, however, in a study of healthy Chinese a non-compartmental analysis showed area under the plasma concentration-time curve (AUC) was lower in subjects receiving naphthoquine with food (64). The lower AUC reflects lower oral bioavailability or higher elimination clearance.

6.1.8 Simulation

Detailed knowledge of the clinical and pharmacokinetic properties of drugs in the target population in which the drug is used, should be the deciding factor in deriving optimal treatment. In the absence, and prior to confirmatory clinical studies, using our detailed models and simulation, along with exposure targets to guarantee parasite cure, we can investigate optimal dosing for different ages. The simulation results in our studies confirmed previous concerns (13) about possible inappropriateness of antimalarial doses administered to children, especially young children, where recommendations were derived from adult effective doses. Kearns et al. 2003 stated that using allometric scaling may work well in children older than 8 years of age and adolescents, whose body composition and organ function approximates young adults (65). However, for young children this may be inappropriate.

No drug exposure targets from the manufacturer to assess optimal dosing in our simulations was found for both drugs amodiaquine and naphthoquine. Therefore, in order to derive optimal dosing, different body weights were evaluated to ensure similar exposures in all weight bands are in

agreement with the exposure in a typical patient (defined here as 50 kg patient). Maximum concentration (C_{max}) was used as a measure of safety, in line with a previous study (66). The optimized dose regimen was constructed to ensure that the median day 7 plasma concentration was not less 80% of the median of the typical patient and that the median C_{max} does not exceed that of the typical patient. Previous studies of antimalarials similar to amodiaquine (19, 60) have also used day 7 concentration as a measure of exposure (11, 66). Bergstrand et al. 2014 (9) compared the association between day 7 plasma concentrations and clinical efficacy in terms of 1-month probability of remaining malaria-free during high malaria transmission. He found that a median expected 1-month parasite-free prevalence of 99% (95% CI: 94 - 100%) if day 7 plasma concentrations of 50 ng/ml was used. Day 7 concentrations correlate with the overall exposure level and was found to be an important predictor of recurrent malaria infection after treatment (11).

Amodiaquine optimal dosing and regimens (Chapter 3)

Simulation of our models for amodiaquine revealed that children of younger age need higher doses than currently recommended in order to secure optimal plasma concentrations. Our models demonstrated that current recommended doses fall short by approximately one third in all of the three children weight groups (< 36 kg). With the new dose recommendation in this thesis, we expect children will receive normal doses as per their biological maturity of the liver. Additionally, we found that higher doses for patient weight over 60 kg would be beneficial. Higher doses for adults may however be impaired by liver and kidney function as a result of aging, however, the inclusion of age effect (as maturation effect in the model) should mean this problem is overcome. This indicates that both weight and age should be considered in drug dosing (67).

A change in amodiaquine dose regimen informed by our recommendations is of great importance as higher drug levels (without increase the risk of toxicity) will prevent patients from the risk of being under-dosed and resulting in failed treatment and may even prevent risk of driving the development of the drug resistance. It is particularly important as concerns for the current dose regimen, especially in children, have already been raised (13), and there is increasing evidence of emergence and spread of artemisinin (ART) resistance (2–4, 68, 69). To our knowledge, this is the first study to demonstrate that amodiaquine exposure is not comparable between patients of different weights. However, similar findings were found for piperaquine (11), an anti-malaria of the same family with amodiaquine (19, 60). Recurrent parasitemia was associated with lower drug exposure for other antimalarial of the same family with amodiaquine (70). In a pooled analysis of efficacy of dihydroartemisinin-piperaquine it was shown that increasing the total dose of piperaquine in children aged 1 to 5 years old from 48 mg/kg to 59 mg/kg would result in successful treatment in at least 95% of these young children (6).

Higher concentrations of amodiaquine are predicted in our simulations to contribute to a more effective therapy and hence reduce the pressure of drug resistance. However, the safety of amodiaquine, as well as its fixed dose partner artesunate, for the optimized dose should be evaluated thoroughly in a clinical trial. With the optimal dose informed by our simulations, the highest dose regimen of amodiaquine is estimated to be 19.3 mg/kg/day, compared to the current maximum target dose of 15 mg/kg/day. The estimated median (range) dose for the optimal dose is 13 (9.2 – 19.3) mg/kg/day, which is also higher than the currently recommended dose of 10 mg/kg/day. Increasing dose of amodiaquine would also require increasing dose of artesunate as these drugs are given in fixed combination. With this, the highest dose regimen for artesunate would be 7.1 mg/kg/day. This increased dose regimen (median (range) dose: 4.9 (3.4 – 7.1) mg/kg/day) of artesunate is higher than the recommended therapeutic dose target of 4 mg/kg/day.

No clinical study was found regarding safety issues when the combination of artesunate + amodiaquine are given at higher dose. However, there is one case study reporting dystonic reaction when the higher dose of drug is given (71). In this study, a female child of three years old, 11.2 kg, homozygous was given 3 times higher dose than the required dose for three days (71). Higher proportion of children developed sinus bradycardia after receiving standard therapeutic dose of 4mg/kg/day artesunate and 10 mg/kg/day amodiaquine (72). Therefore, the optimized dose proposed here should be thoroughly evaluated in patient at risk of cardiovascular disease. In a study by Adjei et al. 2010, one patient developed bradycardia after standard therapeutic single dose of 10 mg/kg amodiaquine. The subject was homozygous for the 2C8*1 variant (71), an enzyme for amodiaquine metabolism. Warranting more safety studies to assess the optimized dose in this group of patients.

The data included in this analysis did not include children weight < 6.5 kg, nor was pregnancy effect included in our models due to the small number of pregnant women. In previous studies pregnancy was found to affect several pharmacokinetic parameter (53, 59, 73). Therefore, dose optimization of this group, as well as younger children than those included in our analysis requires further investigation.

Nevertheless, the present study in Chapter 3, to our knowledge, the largest analysis to date, combining data from 2,920 pharmacokinetic samples from 261 individuals from different target populations with different transmission settings. Our results can be used to inform evidence based optimised treatment in vulnerable young children who account for an estimated 70% of malaria-related deaths (1).

Naphthoquine optimal dosing and regimens (Chapter 5)

The final pharmacokinetic model was used to simulate naphthoquine exposures and maximum concentration at different body weights using the current recommended dose by the manufacturer (Kunming pharmaceuticals, Kunming, China). An evidence-based improved dose regimen was then

developed for young children. Higher doses for young children with weight 29 - 32 kg was recommended in order to achieve equivalent naphthoquine plasma exposures compared to a typical adult patient (50 kg adult). Lower exposures were also observed in patients weighing 46– 49 kg and > 63 kg, leading to a new dosing recommendation in these patients. As people become older, biological organs such as liver and kidney also change due to aging and hence dose increases in adults may be somewhat less representative unless the effect of age has been factored out. Age was not associated with any PK parameter in our study (Chapter 5). Age and weight are in most cases correlated and the effect of age could be explained by the inclusion of allometric scaling on body weight.

This is the first study to demonstrate that naphthoquine exposure is not comparable between patients of different weights. Lower exposure in young children was observed in piperaquine (11, 66), an anti-malaria of the same family with naphthoquine (60, 74). And recently, Hoglund et al. 2017 found that adults with body weights > 60 kg require higher dose of piperaquine than they are currently receiving (66). The revised dose scheme for naphthoquine proposes a minimum dosage of 250 mg as compared to 200 mg (by the manufacturer) for children weighing between 25-32 kg (see table 3 chapter 5). This would give similar exposure to that of typical adult patient and most importantly without exposing them to the risk of toxicity.

The ARCO tablet contains artemisinin and naphthoquine in a fixed combination and the increased naphthoquine doses recommended in this thesis will consequently also increase the artemisinin dosage. With the optimized dose, the highest dose would be 10 mg/kg of naphthoquine phosphate and 25 mg/kg of artemisinin. This increased dose (median (range) dose: 7.8 (6.3 – 10) mg/kg) of naphthoquine phosphate was higher than the manufacturer recommended dose (median (range) dose: 6.9 (5 – 9.5) mg/kg), and for artemisinin (19.5 (15.6 – 25) mg/kg) was higher to the recommended dose of 17 (12.5 – 23.8) mg/kg. Increasing both artemisinin and naphthoquine concentrations should contribute to a more effective therapy, prolonging its useful therapeutic effect and thereby reduce the risk of recrudescence and reinfection, and hence reduce the pressure of artemisinin resistance. However, there are valid concerns to raise in regards the safety of artemisinin at these higher levels as the dose optimization was based solely on simulation of naphthoquine. Assessment of the safety of the proposed higher dose of naphthoquine should also be assessed.

There are several studies examined the safety of combination therapy contains artemisinin and naphthoquine. In a previous study, naphthoquine and artemisinin were given to children 5 – 12 years at higher doses of 9.5 mg/kg and 23.8 mg/kg, respectively. The drugs were well tolerated during 42 days of follow-up, but QTc prolongation observed 4 hours after dosing was determined to be unrelated to the administration of higher doses (75). Laman et al. 2014 studied the safety and tolerability of artemisinin and naphthoquine given as three days regimen comparing with the artemether

lumefantrine. The study involved young children 6 months to 5 years infected with either P. falciparum or P. vivax. The target dose was 8 mg/kg/day naphthoquine and 20 mg/kg/day for artemisinin given for three consecutive days. Overall safety profile for the two drugs was similar except that a higher incident rate of mild short-lived abdominal pain was observed and the QTc increased from baseline to 4 hours after the third dose by a median of 28 milliseconds in the artemisinin-naphthoquine group (76). The prolonged QTc might be the consequence of frequency dosing and not the higher dose of the two moieties and abdominal pain could be because the study was involving very young children but warrants further investigation. In a study of healthy Chinese volunteers, a higher dose of 600 mg naphthoquine/1500 mg artemisinin was compared to 400 mg naphthoquine/500 mg artemisinin, concluding there are no safety concerns for the higher dose naphthoquine/artemisinin (64).

ARCO is formulated for single dose use in contrast to three days of artemisinin drugs currently recommended by the WHO for both efficacy and to delay resistance of artemisinin and its derivative. The main reason for this new formulation was to increase patient adherence. Our analysis revealed that ARCO given as three day or two-day regimen, would result in higher exposure for naphthoquine compared to a single dose regimen, but further pharmacokinetic (PK) and pharmacodynamics (PD) analysis is required to establish the exposure-response relationship for the three days dose regimen of ARCO. There is clinical evidence that when given in three days regimen, ARCO is well tolerated and safe and is more efficacious than when given as single dose regimen(76). In addition, three days of ARCO was shown to be more efficacious than with six doses of artemether-lumefantrine (AL) over three days, after six months of follow up (77). And when it was administered as two doses regimen, ARCO demonstrated higher efficacy compared to single dose with no adverse effect (78) and higher exposure level (62).

There is needed to further explore optimal dosing for patients of weight ≤ 16 kg and pregnant women as our model analysis was based on data that did not include these patients.

6.2 Implication for future research

This is the first study to apply well developed pop-PK models and simulation to create evidence that, amodiaquine and naphthoquine recommended doses in children needs revision. To provide further supporting evidence for decisions on trials and recommended dose changes, a systematic review should be conducted to evaluate all possible clinical trial estimates of efficacy and PK of amodiaquine and naphthoquine dosage in young children. In addition, at the same time, complementary pop-PK models could be developed, or existing models used, to evaluate drug exposure level in young children.

To date, Pop-PK analysis of naphthoquine has been limited. Only one study has been published, undertaken in Papua New Guinea (PNG) reporting lower doses in malaria paediatric patients for naphthoquine. Only children (n = 46, age 5-12 years) were involved in this study (62). Although in our naphthoquine study, we used data from 6-60 years, fewer patients (n = 29) in total were involved in the pop-PK analysis. Therefore, our findings should be viewed as providing more scientific evidence on the existing dosage regimen and the benefits of the new proposed dosage regimen of these antimalarials should be investigated before considered for policy change. Clearly, a pooled Pop-PK analysis of our patients and those in PNG would be the first step to undertake.

Poor compliance by the health professionals to available treatment guidelines and poor patient adherence to the treatment (79) contribute to treatment failure. For example, in a good clinical practice, accurate weights of patients should be taken before dosing, however, in practice this is rarely done. This could result in suboptimal dosing or overdosing. Furthermore, during clinical phase of drug development (phase II or III) usually important subpopulations such as infants and patients with co-morbidities are excluded. Pop-PK and PD studies developed from hospital data (post marketing phase) with high levels of sampling to evaluate the drug exposure-response relationship for children and to allow dose optimization for such vulnerable target population groups is urgently needed.

In our analysis, we assumed thresholds for PD effects for both efficacy and safety in the patients, with a typical adult patient used as reference and assumed to be applicable to younger children. Further research on this assumption is warranted, in particular to address concerns the new proposed dose regimen was not related to the PD aspect of the drug for both safety and efficacy measures, but only exposure. Future studies should aim to assess the safety and importantly the efficacy (the PD), as well as tolerability of the proposed dosage regimen. Pop-PK and PD analysis should then be used to evaluate the exposure-relationship of the proposed dosage regimen.

Further research is required pertaining to the partner drugs, of which many concerns and questions remain as indicated in our main findings. In our analysis only the partner drug with longer half-life (amodiaquine and naphthoquine) were considered and hence their other partner artemisinin/artesunate was not evaluated during dose optimization. There has been significant discussion in the literature that doses of artemisinin and its derivative compounds need to be reviewed. Zaloumis et al 2014 (80) reported from a pooled analysis involving adults and children with severe malaria that, children with weight between 6 and 10 kg, who received 2.4 mg/kg of intravenous (i.v) artesunate, had significantly (p-value < 0.001) lower geometric mean dihydroartemisinin (DHA) exposure (13.7% reduction) compared to the children of higher weight. The author recommended a higher weight-adjusted dose of i.v artesunate in order to obtain similar exposure of dihydroartemisinin, a biological metabolite of artesunate (81), in all children. Similarly, in a study

involved children age 6 months to 11 years with severe malaria who received 2.4 mg/kg intramuscular artesunate, it was found that children with lower weight (6 to 10 kg) had mean reduction of 20.4% (p-value < 0.001) in dihydroartemisinin exposure as compared to children of higher weight (21 to 25 kg) and thus higher dose of artesunate was needed for this young children (8). These would therefore be arguing for the necessity of also reviewing the artemisinin/artesunate doses simultaneously with its partner drugs.

The applications of ACTs and new dosage regimes should also be assessed for other vulnerable populations, for non-falciparum infections, and for use outside of standard treatment, for example in mass campaigns or as routine prophylaxis. Karunajeewa et al. 2010, found significantly lower exposure level of both chloroquine and its active metabolite, desethylchloroquine, when administered as intermittent presumptive treatment in pregnancy for malaria (82). Pharmacokinetic property of artemether, dihydroartemisinin, piperaquine and lumefantrine were found to change during pregnancy (53, 73). Because we could not account for the effect of pregnancy on PK parameters in our analysis, the results of dose optimization we proposed should not be extrapolated to pregnant women and we therefore propose a pop-PK study to evaluate exposure level in pregnant women. Likewise, with the small number of P. vivax included in the analysis, a study to evaluate the effect of malaria species on pop-PK parameters of amodiaquine and assessment of exposure level in children with P. vivax infection is recommended.

Under dosing of antimalarial treatment in children has been shown to associate with treatment failure, risk of recrudescence and anti-malaria drug resistance (6, 10, 83). In this thesis, relationships between lower exposure and the risk of drug resistance was not addressed. However, our work provides a clear path for future research and to use other models of malaria dynamics, and drug resistance dynamics coupled with PKPD models (such as those in this thesis) to explore if and by how much low exposure accounts for spread for drug resistance.

Most clinical studies are completed using vulnerable populations where more intense sampling is considered unethical or even impossible to conduct. In addition, logistical challenges and costs play an additional role in implementing PK studies, and limit sampling numbers. This challenge estimates of PK parameters, they are less precise if an insufficient number of samples is obtained. Optimal design can help determine appropriate sampling schedules that can be used to improve the precision of the PK parameter estimates (84, 85). More importantly, sampling windows are also defined in optimal design, which assures important parameters (e.g. C_{max}) are captured well. The pop-PK models, their parameter estimates, and residual errors developed in this thesis (pooled analysis) can be used for optimal design to aid the best sampling schedule for the new doses we have proposed. This will help

reduce the unnecessary cost of doing large clinical trials and to help overcome the ethical and logistical constraints.

6.3 Implication for new malaria drugs in development

Table 6.1 lists the model and important covariate details of our papers in this thesis and published studies suggesting dose change in children for antimalarial drugs. In total the summary accounts for 8 model-based studies, 5 of which included data of both adult and children. Across all studies both weight and age are important factors for dose changes in children. Age effects explain the maturation of organs or enzyme responsible for drug metabolism, while weight explain the size effect on PK parameters. The non-linear relationship of weight (allometric scaling) and maturation on clearance play a significant role in determining PK parameters estimates and consequently drug dosing in children. Over all the studies there is no clear pattern for effect of maturation on clearance with only two studies that found the effect of maturation on clearance to be important. This might be related to the different enzymes involved in metabolizing drugs, or the effect of age as maturation could be explained by the inclusion of allometric scaling using body weight which in most cases correlates with age. Across the studies, two saw an effect of occasion on bioavailability, which might be related to disease severity. Other markers of disease effect were important in only one of the eight studies (characterised by the effect of haemoglobin). Taken together, these studies provided important information on effects so as to extrapolate from adults to children in the development of new drugs. With aid of population pharmacokinetics, these factors can be simultaneously considered and used as a basis for the current drugs in development like MMV048, KAE609, KAF156, OZ439, and DSM265 (86). Simulation can be used to explore dosing regimens for young children after accounting for those factors and investigating curative dosing during this early stage of development.

In addition, physiologically-based pharmacokinetic (PBPK) models have the potential to improve paediatric malaria drug development as well. PBPK models describe the behaviour of drugs in the different body tissues. Each tissue is considered to be a physiological compartment (87, 88). The effect of growth and maturation on drug PK as well as PD and adverse effects can be handled in PBPK modelling and thus provide support for paediatric dose finding in neonates and infants (88). Given the availability of software such as simcyp (89), the PBPK with the proposed dosing regimen that accounts for the factors determining PK of the drug can then be easily implemented and hence facilitate the process of drug development in children earlier.

6.4 Implication for policy and practice

In 2000 the World Health Organization (WHO) issued new guidelines for the treatment of malaria (90). The WHO is the highest health organization worldwide for which their recommendations on drugs

(use and dosing) based on scientifc finding provides advice and recommendation to member states on changing their policies toward malaria prevention and treatment (91). Through this guideline, most if not all malaria endemic countries changed their policy for malaria treatment from monotherapy to ACTs. The dose recommendation in children from this guideline though was based on the results from dose escalation from adults using allometric scaling.

The pharmacokinetics of drugs depend on body weight but also on maturation processes, change of blood flow of the liver and kidney and these factor do not scale linearly (12). The pop-PK model takes into account both physiological process and the disease status, therefore, the use of pop-PK model in guiding dose optimazation is paramount. As many studies have revealed the evidence on suboptimal dose in children children (5, 8, 9, 11, 66, 80) and with current European pediatric regulation, which aims for the development of safe and of high quality medicine in chidren (92), it is important for the WHO malaria policy advisory committee and regulatory bodies review all the information and create strategy that will help inform guidelines for malaria treatment.

Once scientific based informaton has been gathered (via more research and systematic review) the implementation of these changes at the country level should not cause any obstacles. Malaria policy in malaria endeminc countries has been changing over recent time without major drawbacks by the community (93). The only major challenges being the availability of funds and time to support the implementation of the policy (94), involvement of all key stakeholders early enough (94, 95) and communication on all levels (95). The other challenges include, the readiness of pharmaceutical companies to switch to new policies (94) and the political will (94, 96). In terms of stakeholders, these include national health bodies through to health workers. The use of community health workers (CHWs) has helped implementation of policy at the community level. The community health workers serve as the link between the health authorities or professional and the community they leave and they can undertake activities that lead to improved health outcome (97, 98), through community mobilization and home visiting of individuals (99), of a particular policy or health related meausres.

The optimized dosing proposed in this thesis requires more weight bands than the current recommended dose regimens. More weight bands imply increasing and narrowing the weight range of the current bands. Proposing fewer weight bands results in a wider weight range which in turn results in children receiving suboptimal doses. Fewer weight bands enable easier implementation in health facilities, however, this does not help the patients if there is too high a chance of sub-curative treatment, especially in young children. More weight bands with narrower weight range have also recommended by several other studies of antimalarial drugs, namely artesunate and piperaquine (8, 66). The most problematic path to implementation will lie in the health facility or informal sector where, although they are supposed to measure weights, it is not necessarily standard practice or

possible to accurately measure. In an ideal situation accurate recording of weight would be a routine clinical practice when prescribing medicine as the lack of weight recoding in patient has been associated with narrow therapeutic index antibiotics (100). In clinical practice, we should aim for drugs to be therapeutically effective to all, and with appropriate testing, acceptance in studies, and later emphasize and communication to health professionals on adherence to the good clinical practice and new weight bands should be possible. Despite the challenges ahead to implement changes and dosing on weights, this does not invalidate the scientific evidence in this thesis for ensuring curative ACTs.

Introducing new weight bands requires different dosage and this implies more medications must be ordered by the country. This might raise concerns in regards extra costs the Government will incur, but with the ongoing joint effort to fight the disease worldwide and historical process of drugs decreasing and in some cases more support from the donor organization, this should not pose any challenge. Since under-dosing has been associated with malaria recurrence (101) and recrudescence (11), there is possibility of use of two separate treatments because of a recurring clinical case. One can hypothesize, the average cost of the medication should not increase, and hopefully decrease with overall less treatment and contact with health care. This could be further investigated via economic analysis.

The dose regimen recommended here are based on the current fixed dose formulation and tablet strength and does not take into account the manufacturer processes and limitation. These should be considered in designing new studies and later with the policy bodies so as to not further challenge implementation or decisions. This necessitates analysis to guide the evaluation of alternative dosing, as proposed in this thesis, and how to address and overcome the challenges that might occur such as the issue of patient adherence to the new recommended dosing and system compliance and acceptability to other health stakeholder. This will facilitate more informed policy making.

This thesis did not address system factors leading to loss of effectiveness of malaria treatment, including access, patient adherence and system compliance to obtaining enough drug and following treatment guidelines. These factors will be important to consider in any policy and recommendation changes, and at implementation level focus should also be placed on increasing strength of our health systems.

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This thesis did not address system factors leading to loss of effectiveness of malaria treatment, including access, patient adherence and system compliance to obtaining enough drug and following treatment guidelines. These factors will be important to consider in any policy and recommendation changes, and at implementation level focus should also be placed on increasing strength of our health systems.

6.5 General conclusions

Amodiaquine in combination with artesunate has been adopted as first-line or second-line treatment by at least 25 countries (102), mostly in Sub-Saharan African countries, while naphthoquine in fixed dose formulation with artemisinin is a new generation ACT demonstrating promising results against Plasmodium falciparum and Plasmodium vivax (76, 77). Therefore, dose optimization of these antimalarial is important to maintain their efficacy and reduce the risk of drug resistance. In this respect, pop-pharmacokinetic models were fitted to data from several clinical studies of amodiaquine and naphthoquine. Using simulation, the dose-exposure relationship in patients, in particularly in young children, was evaluated and optimal doses investigated. The main conclusions from the study were:

i. This is the first study to provide evidence of potential suboptimal plasma exposure levels to these anti-malarials and hence confirm previous finding of other anti-malarials that the current dose in young children based on allometric scaling alone is not adequate.
ii. Suboptimal plasma exposure levels of the anti-malarial amodiaquine and naphthoquine in young children were predicted when the antimalarials were given via the current manufacturers' recommended dose regimen. In addition, lower exposure in patients with body weights of more than 60 kg was predicted.
iii. Young children and patients of weight above 60 kg would require higher doses compared to the manufacturer's current dose recommendation. The optimized dose proposed will ensure similar exposure levels over all patient's weight range, result in less treatment failing and potentially reduce the selective pressure of the development of drug resistance.
iv. This optimization is possible to implement in clinical practice, although, assessing the safety of the optimized dose and practical implementation is the next step to ensure the optimized dose is safe, efficacious and well tolerated.
v. Investigation of how the dose optimization affects the exposure of the partner drug is necessary to determine if higher doses are also required for the other moiety in combination. Similarly, exposure-response relationship should be investigated to ensure the safety of these anti-malarial, using the proposed dosing schedule.

Table 6.1 Study and model effects summary of our papers and published pharmacokinetic studies of anti-malaria drugs that have proposed dose optimization for young children

Study and model details included for simulation	Reference							
	Hoglund et al. 2017	Tarning et al. 2012	Zaloumis et al. 2014	Hendriksen et al. 2013	Barnes et al. 2006	Obua et al. 2008	Ali et al. Chapter 3	Ali et al. Chapter 5
Drug investigated	Piperaquine	Piperaquine	Artesunate	Artesunate	Sulfadoxine-pyrimethamine	Chloroquine +Sulfadoxine-pyrimethamine	Amodiaquine	Naphthoquine
Year	2017	2012	2014	2013	2006	2008		
Study population	Adults and children	Children	Adults and children	Children	Adults and children	Children	Adults and children	Adults and children
Analysis used	Pop-PK	Pop-PK	Pop-PK	Pop-PK	NCA	Pop-PK	Pop-PK	Pop-PK
Number of patients	728	236	266	70	307		261	29
Allometric scaling with weight	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes
Effect of age on AUC	-	-	-	-	Yes			
Effect of maturation on clearance	Yes	-	-	-	-	-	Yes	-
Effect of occasion on bioavailability	Yes	-	-	-	-	-	Yes	-
Effect of haemoglobin on clearance	-	-	-	Yes	-	-	-	-

AUC = area under the curve; NCA = non-compartmental analysis, pop-PK = population pharmacokinetic

6.6 References

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7. Personal remarks

In July 2005, just after I graduated from my undergraduate study, I secured a job as statistician and assistant data manager at Ifakara Health Institute, the then called Ifakara Health Research and Development Center. I was allocated to a new established site in Bagamoyo named Bagamoyo Research and Training Center. In 2006, we began our first clinical trial, we had four trials at the same time; RBx11160 (OZ) study, Coartem, Chrolorproguanil Dapsone Artesunate (CDA) and RTS,S malaria vaccine (Mal040). I was very happy to be part of a team that fights malaria disease. I worked really hard and I remember well those nights I spent in the office doing my best to help clinicians sort out queries while waiting for parasitology forms needing to be entered into the database. These forms enabled parasite density results to be calculated for individual patients, as well as the corresponding dose required in order to initiate treatment.

Every time I visited the paediatric ward, I was deeply moved to see the suffering of children due to malaria and other diseases. I was impressed by the clinicians who were busy helping these children. But I could not help being somewhat disappointment in myself that I could do nothing personally to help those children. I regretted why I did not choose to study medicine, because then I would have been able to offer help directly to these children. This motivated me to start searching the internet for a line of discipline that will bring me closer to helping ailing children, and I found “Biostatistics” and how this could be used further in health and medicine. In 2009, while pursuing a MSc in Biostatistics in Belgium, I received 3 hours of lectures on nonlinear mixed effect modelling whereby pharmacokinetic and pharmacodynamics was introduced to me for the first time. I knew then, that my career path was to include pharmacokinetic and pharmacodynamic analysis, in order to directly help children in diseases in studying and optimising treatments.

In 2013, I started looking for a PhD topic in the area of pharmacokinetics and pharmacodynamics, I almost got lost and full of despair after being told that this is a very well-established area and hence, I will not add anything. Thanks to Dr Sasi who introduced me to the idea of optimising dosing in children, I found the right PhD topic, aligned with a health aim I was looking to contribute to for over 7 years. As I’m writing this my heart is full of gratitude and content, and now I’m happy that I will be able to directly help children through my work on dose optimisation. This is the beginning of potentially many encounters with children in fighting against the diseases that affect them, on Allah’s will.

8. Curriculum vitae

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Career Summary

Ali is a biostatistician/Research Scientist with experience in analyzing different type of datasets and have been involved in analyzing clinical trials for evaluating malaria drugs, malaria vaccine and malaria sporozoite human challenge studies. Currently he is PhD student in the department of Epidemiology at the University of Basel & Swiss Tropical and Public Health Institute (Swiss TPH), in Switzerland specializing in Pharmacometrics (PK/PD modeling)

Professional experience and responsibilities

PhD Student, University of Basel 2013 to 2017 (Supervisors: Prof. Dr Melissa Penny, Prof. Dr Tom Smith, Dr Paolo Denti) *SwissTPH Basel and IHI, Tanzania*

Analysis of pharmacokinetic data from different studies data provided by Worldwide Antimalaria Resistant Network (WWARN): "Population Pharmacokinetics of the Anti-malarial Amodiaquine: A Multi-Study Pooled Analysis to Optimize Dosing". Analysis of pharmacokinetic study from Ghana, data provided by WWARN: "Population pharmacokinetics of the anti-malarial amodiaquine with its metabolites (Desethylamodiaquine) in Ghanaians children and adults: Comparing exposure level between children and adults and dose proposal for children". Analysis of pharmacokinetic data from Tanzania (ARCO study from Bagamoyo Research and Training Center, data provided by MMV): "Population pharmacokinetics of the anti-malarial Artemisinin and Naphthoquine in Tanzanian children and adults. Comparing exposure level between children and adults and dose proposal for children" Software used in this analysis: NONMEM, R, Pirana, STATA

Research Scientist (Biostatistician/Pharmacometrician), Bagamoyo Research and Training Center, Ifakara Health Institute (IHI), 2011- Present *IHI, Tanzania*

Statistical analysis plan development on Phase III RTSS Malaria vaccine trial which is a phase III, double blind (observer-blind), randomized, controlled multicenter study to evaluate, in infants and children, the efficacy of the RTS,S/AS01E candidate vaccine against malaria disease caused by *p. falciparum* infection, across diverse malaria transmission settings in Africa. Statistical analysis plan development on Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) study that evaluated the safety and efficacy of Mefloquine as intermittent preventive treatment for malaria on pregnancy. Statistical analysis of PfSPZ challenge study, this is a controlled human malaria infection by intradermal injection of plasmodium falciparum sporozoites (PfSPZ challenge) in Tanzanian adults. Development of statistical analysis plan and statistical analysis of ARCO study (Sponsored by MMV) which evaluated the safety, tolerability & pharmacokinetics of the fixed Artemisinin-Naphthoquine (ARCO®) therapy compared to Dihydroartemisinin Piperaquine to adult patients with P. falciparum malaria. Development of statistical analysis plan and analysis of malaria transmission study which estimated the malaria attack rate and transmission reservoir in the United Republic of Tanzania. Statistical analysis to establish normal range of hematological and biochemistry parameters for Tanzanian adult participating in Phase I Sporozoite vaccine (PfSPZ Vaccine). Statistical analysis of Eurartesim study a multi-country study that evaluated the use of Electrocardiogram to evaluate the safety of ACT in Africa.

Data Manager and Statistician, Bagamoyo Research and Training Center, 2009 - 2011 *IHI, Tanzania*

Data manager and biostatistician on Phase III RTSS Malaria vaccine trial, a phase III, double blind (observer-blind), randomized, controlled multicenter study to evaluate, in infants and children, the efficacy of the

RTS,S/AS01E candidate vaccine against malaria disease caused by P. falciparum infection, across diverse malaria transmission settings in Africa. Data analysis on a phase I malaria vaccine trial (PMal03).

Statistician/Assistant data manager, Bagamoyo Research and Training Center, *IHRDC, Tanzania*
(Ifakara Health Research and Development Center) July 2005 - September 2007
Data management for RBx11160 (OZ) study, Coartem and Chrolorproguanil Dapsone Artesunate (CDA) and RTS,S malaria vaccine (Mal040). Data analysis on prevalence and incidence of Malaria (BMEC Project). Data analysis on effectiveness of Rapid Diagnosis Test (RDT) for malaria. Data analysis on prevalence of malaria for children under 5 year in Bagamoyo for the year 2005 and 2006. Data analysis on references limits for infants, residence of Bagamoyo District.

Skills

- Statistical analysis of multiple clinical trial data sets (clustered randomized control, cross-sectional, longitudinal, time to event)
- Perform linear and non-linear mixed effects modeling
- Perform pharmacokinetic (Pharmacokinetic and Pharmacodynamics modeling, including population-PK analysis of combined data sets
- Software: NONMEM, R, Pirana, Monolix, Stata, Office (word, excel, power point Access)

Grant and awards

2 years scholarship (2007 – 2009) VLIR scholarship to study MSc in Biostatistics in Belgium
9 months scholarship Basel canton scholarship as contribution of expenses in PhD in Basel (Switzerland)

Education

PhD, Epidemiology (Pharmacometrics), University of Basel, Switzerland (July 2013 to June 2017)

MSc, Biostatistics, Universiteit Hasselt, Belgium (September 2007- September 2009)

BSc, Mathematics & Statistics, University of Dar es Salaam, Tanzania (September 2002- June 2005)

Workshops and conferences

Population Approach Group in Europe (PAGE), twenty-fifth meeting, Lisboa (Lisbon), Portugal, 07 – 10 June 2016.

Presentation: Population pharmacokinetics of the antimalarial amodiaquine: Pooling data across different studies to optimize dosing in neglected populations.

Swiss Meeting for Infectious Disease Dynamics (SMIDDY), Haus der Universität (Schlösslistrasse 5, 3008 Bern) 12 September 2014.

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