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Fixed-dose Chloroquine and Sulfadoxine/ Pyrimethamine Treatment of Malaria: Outcome and Pharmacokinetic Aspects

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*This thesis is the basis for a joint degree of Doctor of Philosophy (PhD)
between Karolinska Institutet and Makerere University.*

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**FIXED-DOSE CHLOROQUINE AND
SULFADOXINE/PYRIMETHAMINE
TREATMENT OF MALARIA:
OUTCOME AND
PHARMACOKINETIC ASPECTS**

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ABSTRACT

Background: A pre-packaged fixed-dose formulation of chloroquine (CQ) and sulfadoxine/pyrimethamine (S/P) combination (Homapak) is widely used for the treatment of falciparum malaria in the Home Based Management of Fevers program for Ugandan children. Until the present study, the efficacy, pharmacokinetics and drug interactions of the dose regimen of the product were not known.

Aims: To explore treatment outcome and the pharmacokinetics with fixed-dose CQ+SP combination in uncomplicated falciparum malaria in Uganda.

Materials and methods: In a classical pharmacokinetic study, possible pharmacokinetic interactions between CQ and S/P during co-administration as Homapak were explored and the bioequivalence was determined in healthy volunteers (n=32). Multiple blood samples were obtained on day 0 and up to day 21. Plasma drug levels were assayed using HPLC and classical pharmacokinetic calculations were pursued (**I**). The efficacy of the fixed-dose CQ+SP (Homapak) compared with AQ+SP combination was determined during a clinical trial in children with uncomplicated falciparum malaria (n=183) (**II**). The effects of nutritional status and other host factors at recruitment together with the attained CQ and S concentrations on the treatment outcomes in the children were determined by statistical predictions using regression analyses (**III**). Blood samples collected on filter paper in children (n=83) treated with the fixed-dose CQ+SP were modeled in a population approach using the NONMEM software to determine the exposure (AUC) that predicts cure, from which a proposal for dose modification was made for the treatment of uncomplicated falciparum malaria (**IV**).

Results: Sulfadoxine absorption was more rapid in Homapak ($k_a = 0.55 \text{ h}^{-1}$) than when given as Fansidar + CQ ($k_a = 0.27 \text{ h}^{-1}$, $p=0.004$), but similar when Fansidar was given alone ($k_a = 0.32 \text{ h}^{-1}$, $p=0.03$). Other pharmacokinetic parameters of S, P and CQ were similar when given together or separately, demonstrating bioequivalence of Homapak to reference formulations (**I**). Efficacy of Homapak was tested, and based on the day 14 adequate clinical and parasitological response, CQ+SP (Homapak) had poorer efficacy at 70.9% compared to 97.4% for AQ+SP ($p<0.001$). In those given Homapak, treatment failure rates were much higher (48.2%) for the younger age group given half-strength (HS), than in the older children (18.2%, $p=0.004$) given the full-strength (FS) Homapak (**II**). Among the children given Homapak, stunting was more common (27.7%) compared to underweight (19.3%) and wasting (9.6%), with the mean given doses of CQ and S (mg/kg) and concentrations higher in the FS than HS dose groups. Overall the significant explanatory covariates for cure were day 1 S concentration ($p=0.004$), day 3 CQ concentration ($p=0.037$), and stunting ($p=0.046$) (**III**). By applying a population approach to the response and pharmacokinetics in these children using two-compartmental models the drug exposure (especially AUC_{0-336}) was found to be predictive of response, with the HS dose group having lower AUC_{0-336} values, the majority being below the level predicted for cure. A simulated modified dose, with all children in the age range 6 – 60 months given the same higher fixed-dose CQ+SP would greatly improve the possibility of achieving AUC necessary for cure in at least 95% of the children (**IV**).

Conclusions: The fixed-dose CQ+SP formulation (Homapak) is of good quality. It is however inferior in efficacy compared with AQ+SP combination. AQ could therefore be used as a replacement in combination with SP in the treatment of falciparum malaria where sensitivity patterns permit. To improve response with the fixed-dose CQ+SP a dose modification is proposed by giving all children 6 – 60 months the same higher dose of the formulation.

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LIST OF ABBREVIATIONS

ACPR	Adequate Clinical and Parasitological Response
ACT	Artemisinin Combination Therapy
AIDS	Acquired Immunodeficiency Syndrome
AL	Artemether-Lumefantrine
AM	Artemether
AS	Artesunate
AQ	Amodiaquine
AUC	Area Under the plasma drug concentration-time Curve
BDCQ	Bidesethylchloroquine (also known as didesethylchloroquine)
CDD	Community Drug Distributor
C_{max}	Maximum concentration observed in plasma
CL	The apparent Total Clearance of drug from plasma
CQ	Chloroquine
d	Day(s)
DCQ	Desethylchloroquine
<i>Dhfr</i>	Gene encoding for dihydrofolate reductase enzyme
<i>dhps</i>	Gene encoding for dihydropteroate synthetase enzyme
EANMAT	East African Network for Monitoring Antimalarial Treatment
ETF	Early Treatment Failure
FS	Full-strength formulation of CQ and SP in Homapak
GFAMT	The Global Fund to fight AIDS, Malaria and Tuberculosis
GMP	Good Manufacturing Practices
h	Hour(s)
HBMF	Home-Based Management of Fever (malaria)

HS	Half-strength formulation of CQ and SP in Homapak
IPT	Intermittent Preventive Treatment
K_a	Absorption rate constant
LCF	Late Clinical Failure
LPF	Late Parasitological Failure
MDAQ	Monodesethylamodiaquine
MDCQ	Monodesethylchloroquine
NMCP	National Malaria Control Program
P	Pyrimethamine
PD	Pharmacodynamics
<i>Pfcr1</i>	<i>P. falciparum</i> chloroquine resistance transporter protein gene
PfCRT	<i>P. falciparum</i> chloroquine resistance transporter protein
<i>Pfmdr1</i>	Multidrug resistant gene in plasmodium falciparum
PK	Pharmacokinetics
PPK	Population pharmacokinetics
S	Sulfadoxine
SSA	Sub-Saharan Africa
SP	Sulfadoxine-Pyrimethamine co-formulation
TDM	Therapeutic Drug Monitoring
$t_{1/2}$	Half-life
t_{max}	Time at which the highest drug concentration is attained
V_p	Peripheral volume of distribution
V_c	Central volume of distribution
V_d	Volume of distribution (apparent)
WBC	White Blood Cell count
WHO	World Health Organization

1 INTRODUCTION

In Sub-Saharan Africa (SSA) most parasitic infectious diseases are closely linked to poverty, with their treatment largely dependent on the availability of generic drugs. In malaria, the availability of generic formulations of the easily accessible and affordable chloroquine (CQ) and sulfadoxine/pyrimethamine (SP) has had invaluable impact in the global efforts to control the disease. With these two drugs, millions of lives, especially of children in SSA, have been saved over the last few decades. The emergence of resistance to these two drugs is a tragedy that is reversing the gains that have been made in malaria control. Some equally affordable and readily available alternative therapy is needed. The current artemisinin based combinations therapies (ACT) recommended by the WHO, though highly efficacious, are largely unaffordable to the poor majority that are most hit by the disease.

From a drug-use perspective, knowledge of factors such as the drug quality, parasite susceptibility, drug dosage and pharmacokinetics, and host factor variability are important for the interpretation of treatment outcomes. This thesis explores the clinical efficacy and pharmacokinetics of HOMAPAK – a generic formulation of CQ+SP, packaged as a fixed-dose formulation for community use in the Home Based Management of Fevers (HBMF) program against *Plasmodium falciparum* malaria in Uganda. A formulation about which there was no bioavailability and efficacy data.

1.1 THE MALARIA BURDEN

Malaria is perhaps the most devastating parasitic disease the world has ever known, with over three billion people living under threat of the disease [WHO, 2005a]. The disease is caused by infection with protozoa of the genus *Plasmodium*. In humans, four plasmodial species cause malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, with *P.falciparum* causing the most severe infection. *Plasmodium falciparum* worldwide infects 300 – 500 million people every year, causing the highest burden and mortality especially in the under-fives (20%) of Sub-Saharan Africa (SSA), where nearly one million children die every year from the disease [Snow et al, 1999]. Malaria constitutes 10% of Africa's overall disease burden. It accounts for 40% of public health expenditure, 30-50% of inpatient admissions, and up to 50% of outpatient visits in areas with high malaria transmission [RBM-WHO, 2000]. The heavy toll, among children in the SSA, is the result of a combination of factors such as: 1) the ideal climatic and ecological conditions for the most efficient vector, the *Anopheles gambiae* mosquito, to thrive; 2) the most deadly species of the plasmodium parasites, *P.falciparum*, is also the most common parasites in this sub-region; 3) and it is in SSA where under-development and lack of good-quality healthcare has hindered the control and treatment efforts that had significant impact in other regions of the world [Whitehead et al, 2001]. Furthermore, in highly endemic areas, the severity of infection and mortality from *P.falciparum* is highest amongst pre-school age children due to the insufficient degree of partial immunity in this age group [Hviid, 2005].

In Uganda, malaria is responsible for 25-40% of all outpatient visits at health facilities, 20% of hospital admissions, 9-14% of in-patients deaths with a case-fatality rate of 3-5%, and 23.4% of total discounted life years lost. In the under-fives, malaria accounts for 70% outpatient attendances and nearly 50% admissions in this age group, with malaria specific mortality of between 18-37/1000, which translates in to annual 70,000 – 110,000 deaths in this age group alone. And for the school age children in highly endemic areas of Uganda, it causes absenteeism that affects school performance, with an estimated 60% reduction in the learning ability of the schoolchildren [Uganda-MoH, 2007; UBOS, 2001].

Malaria has been referred to as a disease of poverty [Worrall et al 2005]. Besides causing ill health and death, malaria also greatly affects the social and economic status of the individual, the family, the community and the nation as a whole. For countries whose economies depend on agricultural activities, malaria causes poverty. Since malaria affects people most during the rainy seasons, this interferes with farm activities, thereby creating a vicious cycle of poverty and disease [Worrall et al, 2005]. In some countries with a heavy malaria burden, the disease may account for as much as 40% of the public health expenditure [RBM-WHO, 2000]. In Uganda, a country that depends to a large extent on agro-industrial enterprises, malaria may account for up to 50% of all the man-hours lost, thus affecting production and revenues for the families, industries and the nation as a whole [Uganda-MoH, 2007].

1.1.1 Malaria, malnutrition and immunity

A complex causal interrelationship has been shown to exist between malaria infection, nutritional deficiency and growth. Malaria infection reduces the ability of the affected children to feed properly, subsequently leading to reduced growth and immune status, with the protein-energy deficient children becoming prone to frequent and severe malaria infections [Chandra, 1979]. In an attempt to show a relationship between malaria infection and growth, Hung et al carried out a study to determine whether control of malaria could increase growth in a marginally nourished population of Vietnamese children [Hung et al, 2005]. The study showed that catch-up growth was recorded after control of malaria, where the mean (95% CI) annual increase of height-for-age index (Z-scores) was 0.11 (0.09-0.12) for boys and 0.14 (0.13-0.15) for girls ($P < 0.001$). This growth was attributed to the active malaria control that reduced the parasite carrier rate from 50% at baseline to practically nil in a period of five years. Many reports have linked malaria infection with immunosuppressive disorders, and how the interplay contributes to treatment failure. HIV-associated immunosuppression has been shown to increase malarial fever rates and severity [French et al, 2001; Cohen et al, 2005]. The lack of naturally acquired immunity to malaria (premunition) is known to contribute to the severity of the infection [as reviewed by Hviid, 2005]. Underlying malnutrition has also been shown to increase the frequency and severity of malaria infection [Djimde et al, 2003], and this has been associated with poor treatment outcomes [Caulfield LE et al, 2004; Friedman JF et al, 2005].

1.2 MALARIA TREATMENT

There are two main levels of treating malaria infection. At the individual level, treatment is aimed at cure, prevention of severity and complication, and

minimizing mortality rates. While at the public health level, an additional goal is to prevent emergence and spread of resistance to antimalarials [WHO, 2005]. In order to achieve these goals the most efficacious antimalarials are advocated [Attaran et al, 2004]. For this, antimalarial combinations containing derivatives of artemisinin, also known as artemisinin combination therapies (ACT) have been recommended by the WHO [WHO, 2005]. However, of all the available antimalarials, ACTs are also the most costly (**Table 1**), and this has constrained their availability in the poor countries of SSA [Olliaro and Taylor, 2004]. The WHO has set up a multilateral Global Fund to Fight AIDS, Malaria and Tuberculosis (GFAMT) to assist resource poor nations especially in SSA to access these needed but costly drugs [GFAMT.ORG]. Despite this, access to ACT is still limited in most SSA countries. The WHO does not recommend ACT for use in certain conditions, such as, in the intermittent preventive treatment (IPT) of malaria in pregnancy where SP remains the drug of choice, and in malaria prophylaxis in sickle cell disease where CQ is still used. CQ and SP may however be used for the treatment of *P.vivax* in areas where sensitivity is high, and for the treatment of *P.falciparum* in areas where availability of ACT may be limited [WHO, 2005].

Table 1: Comparative cost of antimalarial drug combinations available in public and private outlets in Uganda

Drug combination	Cost at Public Outlets US\$ ^a	Cost at Private Outlets US\$ ^b
Artemether+lumefantrine (Coartem)	2.40	10.00
Artesunate+Amodiaquine	1.50	7.00
Chloroquine+SP	0.20	1.10

^aSource – UNICEF(a). Procuring supplies for children: Medicines to treat malaria

^bSource – average costs from major pharmacies in Kampala, Uganda.

1.2.1 Treatment failure and parasite resistance

“Treatment failure” in the general context, refers to an unsuccessful treatment outcome from any possible cause, related to the drug, the host and the parasite. “Parasite resistance” on the other hand refers to the ability of the parasite to continue to survive and multiply despite an adequate exposure to the antimalarial drug [White, 2004; WHO, 1973]. Of the various potential causes of malaria treatment failure with either CQ or SP, the most important is resistance-conferring mutations in the parasites [Hyde, 1990; Sibley et al, 2001; Takashi et al, 2001; Sendagire et al, 2005]. The spread, of multi-drug resistant strains of *P.falciparum*, pose an increasing threat to the effective treatment and prophylaxis of malaria. But the advent of molecular studies has made it possible to identify genetic markers of resistance, differentiating “parasite resistance” from other factors that cause lack of clinical response to antimalarial therapy.

Early studies showed that the frequent “treatment failures” that were reported with chloroquine (CQ) in falciparum malaria in Africa, could have been related to poor drug quality and inadequate dosages [Ogwal-Okeng et al, 1998; Basco et al, 1997; Shakoor et al, 1997]. This may lead to the development of severe, complicated malaria, and death, besides causing selection for resistant strains in the otherwise under-dosed patient [WHO, 1986; WHO, 1994]. In the absence of blood drug level analysis, such cases of “treatment failures” as a consequence of sub-therapeutic concentrations may be reported as “parasite resistance”.

Drug concentrations directly account for the observed pharmacodynamic effects. But the attained concentration is attributable to the rate of absorption, distribution in various body compartments, tissue and plasma protein binding, and the rate and extent of metabolism and excretion [Goodman and Gilman's, 11th Ed]. Within individuals, these factors are bound to vary, and it is important to determine the effects of inter-individual pharmacokinetic variability on the therapeutic efficacy of a drug. Factors such as drug dosages also contribute to the observed effects. A recent study showed that individuals that got higher dosages of CQ achieved higher concentrations [Kofoed et al, 2002]. Cure rates increased in such individuals, while the children that got the lower recommended doses registered more failures, and they also had generally lower plasma concentrations.

It has been shown that an important host factor in predicting treatment failure with antimalarials is 'young age' [Staedke et al, 2004]. Thus children living in malarial areas, especially under high transmission pressure, are at higher risk of treatment failure. Such populations may be used as a suitable sentinel population for the monitoring of *in vivo* drug resistance [Fontanet and Walker, 1993; EANMAT, 2003].

1.2.2 Parasite resistance to CQ and SP

Effective control of falciparum malaria has been hampered by the wide spread resistance to the common affordable and easily available CQ and SP [Trape, 2001; EANMAT, 2003]. Uncontrolled use of antimalarials, especially under-dosing, places strong selection pressure on malaria parasites, causing high levels of resistance to develop. As such, since the first case reports in East

Africa, resistance by *falciparum* malaria to CQ (Hess et al, 1983) and to SP [Vleugels, Wetsteyn and Meuwissen, 1982] has progressed unabated in Sub-Saharan Africa.

Polymerase Chain Reaction (PCR) studies of the genomic DNA of *P.falciparum* have been used to identify resistance conferring mutations in the parasite. For CQ, the *P.falciparum* multi-drug resistance gene (*pfmdr1*) [Reed et al, 2000] and the more specific point mutations in the *pfcr1* gene, which encodes for the digestive vacuole transmembrane protein PfCRT that decreases the high-affinity binding sites for the drug [Takashi et al, 2001] have been described (Table 2). For SP, it has been shown that multiple mutant dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*) genotypes are responsible for the resistance of *P.falciparum* to the drugs, respectively [Mutabingwa, Nzila, Mberu, et al, 2001] (Table 2). The presence of mutation at codon 164 of the *pfdhfr* gene provides for high-level resistance [Hyde, 1990]. The development of resistance of *P.falciparum* to SP combinations in the population is attributable to the selection of these resistant mutants that survive under drug pressure, by utilizing alternative metabolic pathways to those blocked by the particular drug [WHO, 1986; WHO, 1994b].

Amidst the chaotic under-dosing, noncompliance and indiscriminate use in all fevers in Africa, the effectiveness of chloroquine as a monotherapy continued for much longer than that of SP. This has been possible because, *P.falciparum* multi-drug resistance –1 (*pfmdr1*) mutations by themselves do not confer resistance [Reed et al, 2000], rather the presence of *P.falciparum* chloroquine resistance transporter (*pfcr1*) mutations are essential, since it takes about eight

to nine *pfcr* mutations in the development of CQ resistance [Wellems and Plowe, 2001]. On the other hand it requires only four sequential mutations in the *pfdhfr* gene, which encodes dihydrofolate reductase, to be sufficient for SP resistance. These four mutations are believed to accumulate much faster than the nine *pfcr* mutations [Sibley et al, 2001].

Table 2: Gene mutations responsible for *P.falciparum* resistance to SP and CQ

Drug	Parasite domains	Points Mutation (codons)	Target parasite protein
Pyrimethamine	<i>pfdhfr</i>	108, 51, 59 and 164 ^a	<i>dhfr</i> enzyme
Sulfadoxine	<i>pfdhps</i>	436, 437, 540 and 613	<i>dhps</i> enzyme
Chloroquine	<i>pfmdr1</i>	N86Y	Transmembrane multidrug P-glycoprotein binding site
	<i>pfcr</i>	72-76 ^b , 97, 220, 271, 326, 356 and 371	Parasite digestive vacuole CQ specific transporter protein

^aMutation at this codon confers high-level resistance to pyrimethamine [Hyde, 1990]

^bK76T Mutation is most important for resistance to CQ [Warhurst, 2003]

pfdhfr = *P.falciparum* dihydrofolate reductase gene

pfdhps = *P.falciparum* dihydropteroate synthetase gene

pfmdr1 = *P.falciparum* multi-drug resistance-1 gene

pfcr = *P.falciparum* chloroquine resistance transporter gene

In areas of intense malaria transmission, interpretation of parasitological outcomes during therapy may be confusing since reappearance of parasites may be wrongly classified as failure when it is actually due to reinfection. Therefore, the WHO recommends the use of PCR for proper classification of parasitological outcomes, differentiating reinfection from recrudescence (therapeutic failure) during treatment [WHO, 2003]. The determination of the parasite surface protein genes *MSP-2* and *MSP-1*, and the genes that code for the glutamate rich protein, *GLURP* have been employed for this purpose [Basco and Ringwald,

2000; Farnert et al, 1997]. Amongst these, the *MSP-2* has been shown to be robust in discriminating between reinfection and recrudescence, and may be used alone for strain differentiation [Cattamanchi et al, 2003; Basco and Ringwald, 2001].

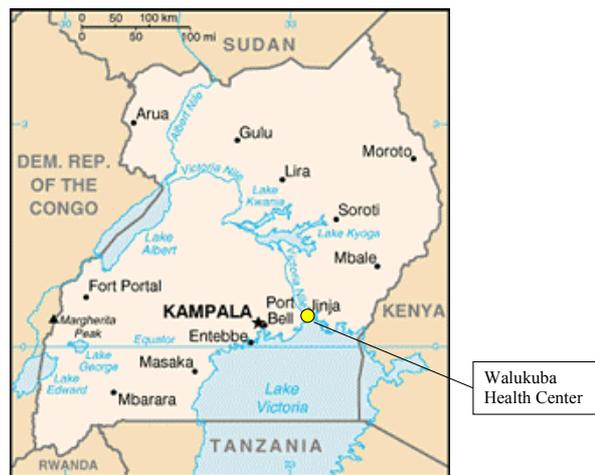


Figure 1: Map of Uganda Showing Walukuba Health Center, Jinja.

1.2.3 Antimalarial efficacy studies in Uganda

Antimalarial trials in urban Kampala area, Uganda, (**Table 3** and **Figure 1**) show that by 1999, CQ monotherapy had a parasitological failure rate of 72%, SP alone had failure rate of 30% [Kanya et al, 2001], while their combination showed a failure rate of 17% in 2001 [Gasasira et al, 2003]. At about the same time, in 2001, a trial conducted in a rural western Uganda showed parasitological failure rate of 10%, 19% and 7% with CQ, SP and CQ+SP respectively [Ndyomugenyi et al, 2004]. Later trials in areas outside Kampala showed increased failure rates with the CQ+SP combination [Yeka et al, 2005; Bakyaite et al, 2005].

Table 3: Recent antimalarial efficacy studies in Ugandan children <5yrs

Site and Time (publication)	Age (# studied)	Drug	Failure rates ^a in the <5yr
Kampala 1999 (Kanya et al, 2001)	53%<5y (n=187)	CQ	paras 72% (22%RIII) clin 54% (23% ETF, 31% LTF)
		SP	paras 30% (5% RIII) clin 11% (5% ETF, 5% LTF)
Kampala 1999-2000 (Staedke et al, 2001)	58% <5y (n=400)	SP	paras 26% (2% RIII) clin 10% (3% ETF, 7% LTF)
		AQ	paras 16% (0% RIII) clin 7% (0% ETF, 7% LTF)
		AQSP	paras 10% (0% RIII) clin 3% (1% ETF, 2% LTF)
Kampala 2000-2001 (Dorsey et al, 2002)	6months-5yr (n=183)	SP	paras 32% (7% RIII) clin 18% (10% ETF, 8% LTF)
		ArtSP	paras 5% (0% RIII) clin 1%
		AQSP	paras 5% clin 1%
Kampala 2001-2002 (Gasasira et al, 2003)	50%<5y (n=416)	SP	paras 30% (5% RIII) clin 15% (5% ETF, 10% LTF)
		CQSP	paras 17% (1% RIII) clin 7% (2% ETF, 5% LTF)
		AQSP	paras 10% (0% RIII) clin 3% (2% ETF, 5% LTF)
Western Uganda Kamwezi 2001 (Ndyomugyenye et al, 2004)	15%<5y n=93	CQ	paras 10% (0%RIII) clin 8%
		SP	paras 19% (0% RIII) clin 0%
		CQSP	paras 7% (0% RIII) clin 0%
Jinja, Arua, Tororo, Apac 2002-2004 (Yeka et al, 2005)	60.3% <5y n=2270	CQ+SP	paras 22% - 46% (PCR adjusted)
		AQ+SP	paras 7% - 18% (PCR adjusted)
		AQ+AS	paras 4% - 12% (PCR adjusted)
Kanungu, Kyenjojo, Mubende 2002 – 2003 (Bakyaita et al, 2005)	70% <5y n=1105	CQ+SP	paras 43% - 73% (PCR adjusted) clin 34% - 67% (PCR adjusted)
		AQ+SP	paras 14% - 38% (PCR adjusted) clin 13% - 35% (PCR adjusted)

^aETF = Early Treatment Failure (formerly RIII = recrudescence rates), LTF = late Treatment Failure, paras = parasitological failure rate, clin = clinical failure rate.

1.2.4 Combination therapy in malaria

Combinations of chemotherapeutic agents can accelerate therapeutic response, improve cure rates and protect the component drugs against resistance. For many years in SSA, the treatment of malaria has been mainly monotherapy with either CQ or SP. However, since the emergence of resistant strains of falciparum malaria [Kean, 1979], monotherapy with these drugs has progressively become untenable [Attaran et al, 2005], and a switch to more efficacious drugs has been called for, their costs notwithstanding [Bell and Winstanley, 2004; WHO, 2005a]. Besides better efficacy, combination antimalarial therapy aims at delaying the spread of drug resistance and thereby prolonging the therapeutic lifespan of the drugs [Bloland et al, 2000]. But as malaria treatment in SSA is characterized by the practice of self-medication [Ruebush et al, 1995], diagnostic accuracy is important to ensure that only proven positive malaria patients are given the regimens, further minimizing the emergence of resistance [Barnish et al, 2004]. For a country like Uganda, the more easily available CQ + SP combination has been used for free distribution in the community in the Home Based Management of Fevers (HBMF) program without laboratory diagnostic backing [Maitland, et al 2004; WHO, 2003b].

1.2.5 Reemergence of parasite sensitivity to chloroquine

Among the known antimalarials there is none that has made such impact on malaria in Africa like chloroquine [Hastings, Bray and Ward, 2002]. At more than 190 tons used per year in Africa alone, CQ benefits have been immeasurable, but it has also been a tremendous driving force in the replacement of chloroquine-sensitive by chloroquine-resistant *P.falciparum* strains [WHO, 1990]. It can still remain an important player in malaria treatment [Ginsburg, 2005] or

make a come-back within the near future following reports from recent field surveys in Thailand [Thaithong et al, 1988], in China [Liu et al, 1995], in Gabon [Schwenke et al, 2001], and lately in Malawi [Kublin et al, 2003; Mita et al, 2003] and Vietnam [Nguyen et al, 2003], which show that the drug appears to regain its efficacy against *Plasmodium falciparum* isolates following withdrawal of its use. In Malawi, Kublin et al found a progressive but steady decline in the *pfcr* genotypes from 85% in 1992 to 13% in 2000, and in 2001 chloroquine cleared 100% of the 63 asymptomatic *P.falciparum* infections with no *pfcr* isolates detected, showing that the *pfcr* phenotypes could not survive through several generations of the parasites and therefore less likely to survive over time [Kublin et al, 2003]. Following this, a 99% efficacy of CQ against *P.falciparum* was reported from a recent trial in Malawi, 12 years after it was withdrawn [Laufer et al, 2006]. Whether this effect is reproducible in other African countries would probably depend on how completely CQ could be withdrawn, a near impossible task for regulatory bodies [Goodman et al, 2004]. It does, however, offer the possibility of reintroducing CQ in a combination therapy after a period of absence and evaluation of regional susceptibility.

1.2.6 Antimalarial drugs

The major antimalarial drugs available for treatment of falciparum malaria in SSA that have been discussed in this thesis include Chloroquine (CQ), Sulfadoxine/pyrimethamine (SP), amodiaquine (AQ), with reference made to the newer artemisinin derivatives such as artesunate (AS), artemether (AM) as the WHO recommended combination therapies.

Table 4: Summary pharmacokinetic characteristics of CQ, AQ and SP after single dose oral administration (mean and range values)

Drug	Pharmacokinetic characteristics				
	Vd (L/kg)	CL (L/h/kg)	t _{1/2β} h (range)	T _{max} h (range)	% Excreted in urine unchanged
Chloroquine	261 (111-411)	0.4 (0.3 – 0.6)	155 (155–336)	2-4	47
Amodiaquine	1.1 ^a 0.3 – 3.6	13 ^a (5 – 57)	4.5 (3.7 – 5.2)	0.5 (0.25-1.0)	5
Sulfadoxine	0.2 (0.1 – 0.3)	1.1 0.7 – 1.7	205 (184-226)	3.9 (3.7 – 4.1)	23
Pyrimethamine	3.1 (2.4 – 3.7)	24 (17 – 36)	107 (96 – 119)	3.9 (3.7 – 4.2)	14

^aAfter I/V injection [White et al, 1987], no oral data available for Vd and CL.

Summary compiled from - Gustafsson et al, 1983; Salako et al, 1987; Frisk-Holmberg et al, 1984; de Vries, Ooterhuis and Boxtel, 1994 for CQ. From – Krishna and White, 1996; White et al, 1987; Winstanley et al, 1987 for AQ, and from – Branes et al, 2006; Edstein, 1987; Weidekamm et al, 1982; Böhni et al, 1969 for SP.

1.2.6.1 Chloroquine (CQ)

Chloroquine is a 4-aminoquinoline, initially synthesized by Andersag in 1934, redeveloped during the Second World War and described as the cheap, safe and very efficacious drug for both treatment and prophylaxis of malaria (Bruce-Chwatt, 1986). It has rapid antipyretic and antiparasitic effects but no gametocidal effects against *P.falciparum*. It has gametocidal effects against the other plasmodial parasites. Although CQ is the cheapest of all the antimalarials (under \$0.10 per adult treatment), it is now estimated that nearly 80% of the world parasite population is resistant to the drug [Ridley, 2002].

Chloroquine is rapidly and completely absorbed when given orally. Oral bioavailability is increased when taken with food [Tulpule and Krishnaswamy, 1982]. Due to extensive distribution into the body compartments, the drug has a large apparent volume of distribution in whole blood [Gustafsson et al, 1983; de

Vries, Ooterhuis and Boxtel, 1994] (Table 4). Its concentration in plasma is 5 – 10 times less than in whole blood as a result of binding to blood cells, especially thrombocytes [Bergqvist and Domeij-Nyberg, 1983; Frisk-Holmberg, 1984; Ducharme and Farinotti, 1996]. Its protein binding ranges from 58% to 64% [Walker et al, 1983; Ofori-Adjeri et al, 1986]. Slightly over 50% of CQ is metabolized in the liver by *N*-dealkylation to its main metabolite, monodesethylchloroquine (MDCQ) with smaller amounts of bidesethylchloroquine (BDCQ) and 7-chloro-4-aminoquinoline. It also undergoes *N*-oxidation to chloroquine *N*-oxide and chloroquine di-*N*-oxide [Ette et al, 1989]. Between 42 % to 47 % of orally administered CQ has been found excreted in urine unchanged. CQ exhibits a multi-exponential elimination pattern with the clinically relevant half-life of CQ being between 6 – 14 days [Gustafsson et al, 1983; Salako et al, 1987]. Longer terminal half-lives have also been reported between in plasma [Frisk-Holmberg et al, 1984] and in urine [Gustafsson et al 1987]. Chloroquine is an old drug, and as such, a Medline search does not yield any recent pharmacokinetic original studies on the drug, except for articles that review its general pharmacokinetics [Krishna and White, 1996; Ducharme and Farinotti, 1996]

CQ is generally safe and any serious adverse effects is usually dose related [Taylor & White, 2004]. Its main common adverse effect is pruritis, which is most pronounced in dark-skinned people, and may be because of the high affinity of CQ for melanocytes [Debing, Ijzerman & Vauquelin, 1988]. In the eye this contributes to retinal toxicity, which may be seen after long-term high-dose therapy such as in rheumatoid arthritis. In the heart, CQ may lead to the fatal but otherwise reversible torsades de pointes [Demaziere et al, 1995], often as a

result of acute poisoning following self-medication in malaria, or during malaria therapy, especially in severe dehydration.

High levels of resistance throughout Africa mean that it can no longer be recommended as a first-line treatment for uncomplicated falciparum malaria [WHO, 2005]. Even though CQ has been declared, “laid to rest”, its use has continued in many countries in Sub-Saharan Africa such as Congo, Ghana and Uganda [Nsimba et al, 2004; Koram et al, 2005; Yeka et al, 2005]. Even in countries where it has been officially replaced as the policy drug, CQ is still freely available and used [Goodman et al, 2004]. In some areas, its use has been recommended in combination with other antimalarial drugs [Ndyomugenyi et al, 2005, Sendagire et al, 2005], while in Guinea-Bissau its use as a monotherapy but higher dosages has been recommended [Kofoed et al, 2002]. Differential use in population sub-groups has also been considered, especially in adults where CQ remains effective due to the partial acquired immunity from malaria episodes [Ginsburg, 2005].

1.2.6.2 Amodiaquine (AQ)

Amodiaquine (AQ) is a readily available and inexpensive 4-aminoquinoline (at \$0.15 per adult treatment), chemically related to CQ but often effective against CQ resistant strains of *P.falciparum* [WHO, 2001]. AQ is rapidly absorbed when given orally with a very short terminal half-life (Table 4) in both malaria patients and healthy volunteers respectively [Krishna and White, 1996; Winstanley et al, 1989; Winstanley et al, 1990]. It undergoes rapid *N*-dealkylation to its active metabolite, monodesethylamodiaquine (MDAQ), with peak concentration (19 fold higher than those of the parent drug) reached in whole blood within 2.3 ± 0.5 h and

in plasma within 3.4 ± 0.8 h, and eliminated much more slowly over a longer period of time [Winstanley et al, 1987].

Over the years, AQ use declined as severe adverse reactions, such as agranulocytosis and hepatotoxicity, were reported during chemoprophylaxis (Hatton et al, 1986; Neffel et al, 1986). The WHO recommended that AQ should not be used for prophylaxis (WHO, 1990), but later amended that it could be used for malaria therapy if the benefits out-weighed the risks [WHO, 1993]. When used therapeutically, no severe adverse effects were reported [Olliaro et al, 1996; WHO, 2001; Bloland, 2003]. Within Africa, AQ alone or in combination with SP has been shown to be more effective than CQ alone or CQ+SP in the treatment of uncomplicated falciparum malaria [Staedke et al 2004; Oduro et al, 2005]. Some SSA countries, such as Rwanda and Zanzibar, have already adopted AQ+artesunate (AS) combinations as first-line therapy. In Uganda AQ+AS is used as an alternative when artemether-lumefantrine (Coartem®) combination is not available [EANMAT, 2003].

1.2.6.3 Sulfadoxine–pyrimethamine (SP)

Although a combination of two drugs, SP is not considered a combination therapy as the component drugs act on the same target, the parasite folate biosynthesis. SP is cheap at about US\$ 0.2 per adult dose and easily available. Taken as a single oral dose, both S and P show similar pharmacokinetic profiles as shown in Table 4.

Following oral intake in healthy volunteers, S has fairly rapid absorption [Weidekamm et al, 1982; Edstein, 1987] (Table 4). A longer t_{max} (13.5h) was

found in children with falciparum malaria [Winstanley, et al, 1992] with the authors suggesting that the rather delayed t_{max} might have been due to changes in the kinetics of the drug during malaria. In healthy volunteers, after single dose, it has been shown to have a long elimination half-life [Weidekamm et al, 1982; Edstein, 1987] (Table 4). Equivalent values (7.7 days equivalent to 184.8h) were also found after repeated administration in healthy volunteers [Hellgren et al, 1990]. In children with falciparum malaria it was found to be 116h (Winstanley et al, 1992). Sulfadoxine has a rather low V_d [Edstein, 1987; Wang et al, 1990] (Table 4).

Pyrimethamine after oral intake has a similar t_{max} to that of S (Table 4) in healthy volunteers [Edstien, 1987; Edstein et al, 1990; Weidekamm, 1982]. A longer t_{max} (12h) was also shown in children with falciparum malaria [Winstanley, 1992]. Its elimination half-life is relatively short compared to that of S (Table 4) [Edstein, 1987; Edstein et al, 1990; Weidekamm et al, 1982]. Similar $t_{1/2}$ values (mean of 3.9 – 4.2 days) were found by others [Hellgren et al, 1990; Wang et al, 1990]. The oral $t_{1/2}$ in falciparum-infected children was found to be 81h (Winstanley et al, 1992). Its mean V_d is larger than that of S (Table 4) [Edstein, 1987; Wang et al, 1990].

Sulfadoxine/pyrimethamine has been used to replace CQ as a first-line treatment for uncomplicated malaria in many countries in Africa, with Malawi setting the trend in 1993 [Trape, 2001; Kassa et al, 2005; Eriksen et al, 2005].

Unfortunately, resistance has quickly developed, facilitated by its long half-life. In Uganda, SP combination with CQ was adopted in June 2002 as a first line antimalarial policy drug [EANMAT, 2003]. Following efficacy studies in Uganda,

recommendations were made for the withdrawal of CQ and to replace it with AQ in this combination [Kanya et al, 2001], as the combination of AQ with SP was demonstrated to be more effective [Olliaro et al, 1996; Bloland, 2003]. SP however remains the drug of choice in the intermittent preventive treatment (IPT) of malaria in pregnancy [WHO, 2006].

1.2.6.4 Artemisinin combination therapy (ACT)

By borrowing a leaf from the standard practice in the treatment of HIV/AIDS and tuberculosis, combination chemotherapy, using two or more antimalarials with different mechanisms and sites of action, is proposed as a means of slowing the process of development of resistance. Artemisinins have been recommended as ideal drugs for use in combination therapies; this treatment is known as artemisinin combination therapy (ACT). Artemisinins are sesquiterpine lactones characterized by possession of an endoperoxide ring responsible for its antimalarial activity. They have the broadest activity against malaria parasites, from the ring stage to early schizonts, and cause the fastest decline in parasite numbers of all the antimalarial drugs. Artemisinins, however, have very short half-lives and as a monotherapy must be taken for at least 7 days. In ACT, the artemisinin is taken for 3 days, significantly reducing the parasite numbers and leaving the remaining parasites to be killed by the second drug (or the host immune system) [WHO, 2006]. There have been reports on possible neurotoxicity due to artemisinin, although this seems unlikely in the current doses employed in malaria therapy [reviewed by Toovey S., 2006]. The WHO recommends that artemisinin should only be used in combination. At present the following combinations are associated: artemether-lumefantrine (AL),

artesunate+amodiaquine (AS+AQ), artesunate+mefloquine (AS+M) and artesunate+sulfadoxine/pyrimethamine (AS+SP) [WHO, 2006].

In Thailand, the combination of mefloquine with artesunate was introduced in 1994 and resulted in sustained high cure rates, a reduction of the *in vitro* mefloquine resistance and a sustained decline in the incidence of *P.falciparum* malaria in that area [Nosten et al, 2000]. Whether such effects would be seen in Africa, where transmission intensity, acquired immunity and treatment practices are very different, is not known and is the subject of much debate [Duffy and Mutabingwa, 2004].

1.3 PHARMACOKINETIC APPROACHES

Drugs are commonly administered by the oral route, after which they must undergo absorption, distribution, metabolism and then excretion. These processes constitute what is described as pharmacokinetics. The rate at which these processes occur depend on many factors, some of which may be ascribed to the pharmaceutical properties of the drug and others to host [Rowland and Tozer, 1995].

1.3.1 Traditional vs. population approach to pharmacokinetics

Pharmacokinetics refers to the mechanisms and extent of absorption, distribution, and elimination of the administered drug [Rowland and Tozer editors, 1995]. It is related to adjustable elements such as dose, dosage form, frequency, and route of administration that affect the drug level-time relationships in the body. More importantly these elements influence the *Pharmacodynamics*, where the drug concentration at the site(s) of action is related to the response produced. These relationships have for long been determined through the

traditional PK studies (Table 5). The results are then extrapolated to the sick population without specific studies in this group.

A realistic evaluation of the kinetics of the drugs in disease states necessitates studying them in patient populations, an approach referred to as population pharmacokinetics [Beal and Sheiner, 1982; Kauffman and Kearns, 1992] (Table 5). This approach allows for few samples to be taken from each individual within the patient population, and then applying analytical methods that allow for modeling of sparse data to describe the population kinetics of a drug [Arrons, 1991; Whiting et al, 1985]. The technique facilitates investigation of factors that cause variation in drug concentrations and hence lead to better prediction of dose and/ or response in individuals receiving the drug in the future. Statistical methods developed for this purpose include, the nonlinear mixed effect modeling (NONMEM) [Sheiner et al, 1972], which is based on the assumption that the kinetic parameters are normally distributed. Its biggest weakness is that, being a parametric method, outliers are often excluded as they are considered to cause biased estimates. Another analytical method is the nonparametric maximum likelihood (NPML) method [Mallet, 1986], which does not require previous assumptions on the parameter distribution and takes into account the outliers.

Table 5: Differences between classical vs. population pharmacokinetic studies

Practical Aspects	Classical PK studies	Population PK studies
Design	Prospective and highly controlled	Both prospective and retrospective or part of TDM and may/may not be controlled
Subjects	Usually adult healthy volunteers	Specific patients populations (include vulnerable subjects like children)
# Of participants	Often restricted and few	Often as many patients as can be recruited
Blood Sampling	Multiple intense per subject	Sparse - Any number (even one) per patient

TDM=Therapeutic Drug Monitoring

1.3.2 Bioavailability and bioequivalence

During medication, one of the pharmacokinetic parameters that concern the clinician most is *bioavailability*. This is the fractional extent to which an administered drug reaches the systemic circulation, where it gains access to its site of action [Goodman& Gilman's 11th Ed]. For a given dose, the bioavailability is therefore determined by the amount, the rate and extent to which it is absorbed and eliminated (first-pass-effect). *Relative bioavailability* on the other hand is a ratio of the bioavailability of different dosage forms or different routes (usually by comparison of the oral or other routes against the intravenous route) of administration of the same drug. It may also involve comparison of the bioavailability of a drug administered during different disease conditions.

For resource limited countries especially in SSA, where treatment policies depend to a large extent, if not wholly, on the use of generic drugs [Brundtland,

2002; Quick et al, 2002], this ratio becomes very important when different formulations of the innovator drugs are to be evaluated for quality. That is, once the patent for a specific drug expires, anybody may manufacture formulations bearing the common scientific name of the patented drug, provided that it is *bioequivalent* to the innovator drug. The product, thus manufactured, is called a generic product. Its manufacture should be guided by the Good Manufacture Practices (GMP) code [EMA, 2001].

The major concern with generic products is the ability of a patient to exchange one product for another (*bioequivalence*). Two products are considered bioequivalent if the concentration-time profiles are so similar that they are unlikely to produce clinically relevant differences in either therapeutic or adverse effects [Rowland and Tozer, 1995]. For a new product to be considered bioequivalent, the relative bioavailability ($AUC_{\text{test}}/AUC_{\text{reference}}$), should give a ratio between 0.80 – 1.25 within the 90% confidence interval [EMA, 2001].

1.3.3 Pharmacokinetics in undernourished malaria infected children

Studies on quinine disposition in undernourished children with malaria showed inconsistent pharmacokinetic findings. One study found increased plasma concentration of quinine in malnourished children [Pussard et al, 1999], while other studies found reduced absorption, lower concentrations and reduced elimination half-life [Treluyer et al, 1996; Salako et al, 1989]. Another study that investigated the pharmacokinetics of CQ in the undernourished and the healthy adult volunteers found no significant differences in the pharmacokinetics of CQ between the two groups [Tulpule and Krishnaswamy, 1982]. The study

concluded that the undernourished do not run the risk of therapeutic failure or toxicity with chloroquine.

During the decades of use in the treatment of malaria, not much is known about the pharmacokinetics of chloroquine or SP in undernourished malaria infected children. It is therefore possible that interindividual (pharmacokinetic) variations in drug concentrations that have been reported [Rombo et al, 1987; Hellgren et al, 1989] could partly be the consequence of host anthropometric variations.

1.4 POLICY CHANGES IN MALARIA TREATMENT

Change in drug policy for the treatment of malaria is a major undertaking for most SSA countries. This stems from the fact that the process is complex, requiring evidence from research about the need for change (from the level of resistance against the current drug), then formulation of the policy change by the concerned stakeholders in consultation with all stake holders, and finally the political implementation of the policy with the implied financial commitments [Williams et al, 2004]. As such, the cost of treatment has a disproportionate influence on the decision-making process [Fevre and Barnish, 1999].

Guidelines issued by the WHO on when to change a drug policy often overlap the decision-making process in most countries, for example, from 25% parasitological failure recommended in 2003 [WHO, 2003], to the current 10% parasitological failure, PCR adjusted for reinfections by day 28, recommended in 2006 [WHO, 2006]. What this means is that, countries that had made policy changes based on the 2003 guidelines would need to make new changes often before full implementation of the previous policy change.

For a low-income country like Uganda, the complexity of policy change is best understood by a brief overview of the chronology of the country's antimalarial policy changes in the recent years to date. In 2000 the WHO recommend ACT as first line against *P.falciparum* malaria [WHO, 2000b; WHO, 2001a]. Following the Heads of Governments conference in Abuja April 2001, the National Malaria Control Program - Uganda (NMCP) announced change in the malaria treatment policy from CQ monotherapy to CQ+SP combination for the treatment of uncomplicated malaria [MoH-Uganda, 2002]. This decision was based on the result of studies that showed improved efficacy with the combination of CQ+SP as compared to the single therapies, and that the combination could protect SP. The combination locally known as Homapak was thus officially launched in June 2002. Homapak is pre-packed age-based fixed-dose generic formulation of CQ+SP, specifically for the treatment of uncomplicated malaria in children in the HBMF program. The free distribution of Homapak started as a pilot in a few districts.

By the time the clinical study in this thesis was conducted (July –November 2004, nearly two years after the launch), the free distribution of Homapak had not yet been implemented in Jinja district (**Figure 1**). At about the same period studies on the efficacy of CQ+SP started to indicate high failure rates with the combination, especially in the urban areas of the country [Dorsey et al, 2002; Gasasira et al, 2003]. Then in June 2004, a new ACT policy was announced by the NMCP-Uganda for treatment of uncomplicated malaria, with a launch in June 2005. However, it was not until April 2006 that the first batches of AL (Coartem®) were supplied to major health units (hospitals and health centers). So far, there

is no data on the suitability and overall effectiveness of ACT when given in the HBMF at home.

Though ACT is efficacious in *P.falciparum*, the risk of resistance developing when given without proper diagnosis may jeopardize the ACT policy. Currently there are on going studies in several SSA countries, including Uganda, to find out if the community distribution of ACT is feasible, acceptable, safe and effective [Nakazibwe, 2006]. In the mean time, policy statements from the Ministry of Health, Uganda, gives no recommendations on the use of ACT in the HBMF program [MoH- Uganda, 2006], and Homapak continue as the drug therapy in the program. It is against this background that the studies discussed in this thesis were conducted.

1.4.1 Homapak and Home-Based Management of Fever (malaria) Program in Uganda

Homapak is the local name given to the pre-packed age-based fixed-dose generic formulation of CQ+SP manufactured Kampala Pharmaceuticals Industries Limited (KPI Ltd, Uganda). It is specifically made for the HBMF program in Uganda for free distribution in the community. Its packaging has age-specific dosing schedules. “Half-Strength” (HS) dose is for the younger children (2 months to 24 months), and the “Full-Strength” (FS) dose for the older children (2 to 5 years) (*III*). The terms “Half-strength” and “Full-strength” refer to the fact that the packaging contain “half” or the usual “full” tablet strength, respectively. Records available from the Uganda National Drug Authority (the national drug regulatory body) show that the formulation fulfilled all physiochemical properties (BP specifications for weight uniformity, content, dissolution time, disintegration

time, friability and crushing strength) required for registration. This paved way for its subsequent deployment in the HBMF program. Community appointed volunteers, commonly known as Community Drug Distributors (CDD), are responsible for the distribution of Homapak. The CDD(s) are usually individuals with no formal medical training, but they liaise with the local health units for supplies [Killian et al, 2003; Nsungwa-Sabiiti et al, 2005]. Colour codes on Homapak packages, red for the HS and green for the FS, guide the CDD(s) and mothers or guardians in choosing the correct dose for a child of a particular age.

The WHO Roll Back Malaria (RBM) program recommends the presumptive prompt treatment of malaria in children within 24 hours of onset of fever in the home setting, a strategy called the HBMF program [WHO, 2000a]. In line with the recommendation, the National Malaria Control Program (NMCP) of the Ugandan Ministry of Health adopted this strategy that relied on the prompt and free distribution of Homapak to all children, under-five years of age, in the home setting.

1.5 RATIONALE FOR THE STUDIES

At the time the studies in this thesis were proposed, there were no bioavailability or bioequivalence data on Homapak, the designated policy drug for the HBMF program in Uganda. As such, these knowledge gaps required PK studies on the drug. PK investigations however, require heavy investments in technological and human resource capacity, both of which have been lacking at Makerere University or the country at large. Despite these limitations, the possibility to perform PK studies at Makerere would therefore rely on human capacity building; procurement of equipments and some pioneering work. With no prior experience in the field,

conditions under which this kind of research can be performed in resource-limited settings presented challenges that needed to be coped with. This thesis is the result of pioneer works in pharmacokinetic studies at Makerere University, made possible by exploring research collaboration opportunities between Karolinska Institutet, Sweden and Makerere University. The impact of which can be described as the birth of clinical pharmacokinetics at Makerere University.

Chloroquine plus sulfadoxine/pyrimethamine combination, though progressively failing as treatment option for falciparum malaria, are still widely used in Uganda and other Sub-Saharan African countries. The search for a suitable alternative to CQ in the combination with SP required comparison with an equally cheap readily available alternative such as AQ+SP in a clinical setting. With the reported high failure rates with CQ and SP in Uganda bearing in mind regional differences presented in Table 3, the complexity of treatment failure in malaria should be seen in the light of all factors, including resistance conferring genes in the parasite, the drug quality and bioavailability, and other associated patient factors that contribute to the outcomes of therapy. Treatment failures with CQ and SP probably arose from the use of poor quality drugs [Behrens, Awad, Taylor, 2002; Ogwal-Okeng, Owino, Obua, 2003], and failure to follow recommended doses. These drugs have been the backbone of malaria chemotherapy in SSA. Under or over dosing with CQ and SP or their combination, especially in children, has been reported [Tumwikirize et al, 2003; Nsungwa-Sabiiti et al, 2005]. While the unit-dose packaging of antimalarials may improve treatment adherence, there is little evidence to say if it improves the effectiveness of treatment [as reviewed by Orton and Barnish, 2006]. With the introduction of Homapak for malaria treatment in children in the community, it

was necessary to describe the pharmacokinetics of this fixed-dose combination therapy first in healthy volunteers (for quality and safety reasons), and subsequently in children with uncomplicated malaria.

Little is known about the criteria used for the determination of the dosing schedule of Homapak. This is probably due to the fact that pharmacokinetic studies of the drugs in children are not very common. Lack of pharmacokinetic data on CQ and SP in children with uncomplicated falciparum malaria required exploration in this patient population. Therefore, there was need to determine the predictors of treatment outcomes by applying a population approach to the pharmacokinetics of the Homapak in malaria treatment, findings from which may form the basis for recommending dose designs in fixed-dose combinations in malaria.

1.6 THE STUDIES

1.6.1 Overall aims of the study

The thesis is an explorative evaluation of the pharmacokinetics, interactions and efficacy of the fixed-dose CQ + SP combination (Homapak) used to treat falciparum malaria in Uganda.

1.6.2 Specific objectives

The specific objectives were to:

1. Determine the bioequivalence of CQ and S/P in the pre-packaged fixed-dose formulation (Homapak) combination compared with that of GMP made CQ and S/P controls in healthy adult subjects. *(I)*
2. Explore possible pharmacokinetic interactions between CQ, S, and P when administered concurrently. *(I)*
3. Determine the clinical and parasitological efficacy of fixed-dose CQ + S/P formulation (Homapak) compared to Amodiaquine (AQ) + S/P combination in the treatment of uncomplicated falciparum malaria in Ugandan children. *(II)*
4. Explore the importance of host factors and drug concentrations for the treatment outcomes with fixed-dose CQ + S/P (Homapak) in uncomplicated falciparum malaria in Ugandan children. *(III)*
5. Determine the population pharmacokinetics of chloroquine and sulfadoxine in Ugandan children treated with fixed-dose CQ+SP during uncomplicated falciparum malaria infection. *(IV)*

2 MATERIALS AND METHODS

2.1 PARTICIPANTS AND DESIGN FOR HEALTHY VOLUNTEERS STUDY (I)

In the pharmacokinetic study in healthy volunteers (carried out in July - August 2003), 32 adults were recruited from paramedical, nursing and medical students between 20 and 30 years of age. Screening for history of antimalarial intake in the last three months prior to the study, current medications and proven general health was important for recruitment. Volunteers were randomized to take single oral doses of the generic fixed-dose CQ+SP (Homapak), GMP SP, GMP CQ or combination of GMP CQ+SP, without crossover due to the very long elimination half-life of the drugs. Venous blood plasma samples were collected from each volunteer in heparinized vacutainer tubes at intervals spread over a period of 21 days. Multiple sampling on the first day of medication was made possible by the use of indwelling intravenous catheters. The assays for chloroquine, sulfadoxine and pyrimethamine levels were done using high-pressure liquid chromatography (HPLC) methods as previously described [Bergqvist et al, 1985 and Minzi et al, 2003] (I).

2.2 PARTICIPANTS AND DESIGN FOR THE CLINICAL AND PHARMACOKINETIC STUDIES IN CHILDREN

In the clinical trial, an efficacy study of Homapak (CQ+SP) compared to AQ+SP was carried out in children with uncomplicated falciparum malaria based on the WHO protocol [WHO, 2003], with a follow up of 28 days (II). Children between 6 months and 5 years consecutively presenting with a suspicion of malaria were screened and recruited if they had uncomplicated falciparum malaria. The

primary endpoint was day-14 clinical and parasitological efficacy (Adequate Clinical Parasitological Response, ACPR) using the per-protocol analysis for the children who completed the study, and the intention-to-treat (ITT) analysis for all enrolled children. Treatment outcome was classified according to the WHO, 2003 protocol (*II*). Secondary outcomes were the mean parasite clearance time (PCT), parasite recrudescence by day-28, gametocyte carriage rates, Hb changes, and mean fever clearance time (FCT). Assuming 95% d14 ACPR for AQ+SP (Table 3), the minimum number required to detect a 15% difference in response between AQ+SP and Homapak at 95% level of confidence and power of 0.80, was 73 children in each arm [Huitfeldt, 1986].

During the clinical trial, additional information on age and sex, measurements of weight, height and mid-upper-arm circumference (MAC) were done, and from these host anthropometrics were obtained (**Paper III**). These were modeled to identify independent explanatory covariates for cure or failure with the fixed-dose CQ+SP formulation. Capillary blood samples applied on filter paper were sampled over 14 days and were used for the analysis of CQ and S separately (*III & IV*). Blood smears on microscope slides, taken for parasite counts and gametocyte carriage, were followed up for 28 days, with additional samples taken on filter paper on days 0 and 28 for purpose of genotyping to differentiate re-infection from recrudescence using polymerase chain reaction (PCR) analysis (*II*). Additional filter paper samples of 100µl capillary blood were taken from recruited children at different time points within the first eight hours after medication. These samples provided data points that were pooled for the population pharmacokinetic evaluation of chloroquine and sulfadoxine (*IV*).

There were therefore several sampling occasions, which required more human resources. Thus a larger number of field assistants than was previously planned was put in place, with the attendant financial implications for which adjustment had to be made from within the limited funds. Logistically making frequent sampling in children is no ordinary task provisions have to be put in place to ensure their continued co-operation. This included providing the children with cookies or sometime just a lollipop during sampling, while the parents/caretakers were given transport refunds for every visit made.

2.3 LABORATORY METHODS

2.3.1 Determination of haemoglobin concentration

Capillary blood from fingertips was obtained for the determination of haemoglobin concentration (Hb) during assessment for inclusion and by day 28 of follow up. The Hb concentrations were determined using a portable Elaehaem1 Haemoglobinometer (Lovibond®, John Morrison Scientific, Australia).

2.3.2 Thin and thick blood smears for parasite counts

The thin and thick blood smears were stained using freshly prepared 2% Giemsa. From the thick smears, parasite density was determined by counting the number of asexual parasites against 200 white blood cells (WBCs). The parasite count per μl was calculated as: $40 \times \frac{\text{number of parasites}}{200 \text{ WBCs}}$, assuming a normal WBC count of $8000/\mu\text{l}$. A smear was considered negative after examination of 100 high-power fields. The thin smear was used for species determination. Two experienced microscopists screened the smears independently, and if discrepancy in parasite count was less than 10%

between the two microscopists, the average was taken. In case of more than 10% discrepancy, a third independent microscopist was used to confirm the counts. For quality control, ten slides out of every hundred were randomly selected and reviewed independently by the third senior microscopist. Overall, variation in the counts ranged from 1% - 13% of the recorded counts (*II*).

2.3.3 Differentiation of reinfection from recrudescence

Paired samples from day 0 (d0) and the day of parasite reappearance were subjected to the nested PCR characterization of the merozoite surface protein-2 (MSP-2) genes using the IC3D7 and FC27 allelic families as described by Cattamanchi et al, 2003. The outcomes were defined as recrudescence if at the time of reappearance, the subset contained half or more of the alleles that were present at d0, and as reinfection if more than half the alleles found on the day of reappearance were not present on d0 [Cattamanchi et al, 2003] (*II*).

2.3.4 Determination of blood drug levels from the clinical trial

2.3.4.1 Pharmacokinetic sampling

Blood samples from finger-pricks (100µl) were collected over a period of 14 days using precision capillary tubes, and dried on Whatman No. 1 filter paper, which were then stored at room temperature. Additional post-dose samples were collected between 0 to 8h, with each patient supplying at least one data point. The day 1 and day 2 samples were collected just before the repeat CQ dose. Blood samples on the other days were collected at about the same hour of day for the individual participant. The filter paper samples were stored for between 3-6 months before analysis.

2.3.4.2 Bioanalytical methods

For CQ, filter paper samples were cut into pieces placed in 15 ml polyethylene tube to which 0.5ml of diethylamine (DEA) 0.5% in water, 25 μ l of 2.9 μ mol/L 4-(4-dimethylamino-1-methylbutylamino)-7-chloroquinoline as the internal standard (I.S), 2ml of potassium hydroxide (1 mol/L), and 7ml of di-isopropyl ether were added. Extraction was on a reciprocal shaker for 20 min. and then centrifuged for 10 min. at 3500 x g. The organic phase was transferred to another 8ml polypropylene tube and back extracted using 150 μ l of a phosphate buffer, pH 2.5. 130 μ l of the organic phase was aspirated and injected into the chromatographic system. The mobile phase consisted of a mixture of methanol/phosphate buffer/perchloric acid (at 250:747.5:2.5, v/v). Elution was at flow rate of 1.5ml/min [Minzi et al, 2003]. The limit of quantification for CQ was 48 nmol/L, with the inter-assay coefficient of variation at 2.9%.

For S, after cutting the dried spot into pieces they were placed in polypropylene tubes to which 1.5 ml of 0.1mol/L sodium hydroxide, 100 μ l of 750 μ mol/L sulfamethoxazole (IS) and shaken for 15 min. To which 0.25 ml of 1 mol/L zinc sulphate was added and shaken for 15 min, and centrifuged at 1000 x g, from which. 20 μ l of the clear supernatant was injected into the chromatographic system. The mobile phase consisted of acetonitrile/phosphate buffer, pH 3.0 (20:80, v/v) [Bergqvist et al, 1987]. The limit of quantification for S was 14.5 μ mol/L, with the inter-assay coefficient of variation at 4.4%.

2.4 PHARMACOKINETIC CALCULATIONS

The pharmacokinetic parameters in the healthy volunteers (*I*) were calculated and modeled using WinNonlin version 4.1 software (Pharsight Inc., Mountain

View, CA, USA). The two-compartment model was used to calculate the absorption rate constant (k_a) for chloroquine, while the one-compartment model was applied for sulfadoxine and pyrimethamine. Half-life ($t_{1/2}$) was calculated from the terminal slope (λ) of the concentration-time profile, and the area under the curve (AUC) calculated using the trapezoidal rule. Clearance (Cl) and volume of distribution (V_d) were normalized by body weight. Where wide variances in the values were encountered the non-parametric, Kruskal-Wallis test was applied. For normally distributed values the independent t-test was applied. Statistical significance was assumed only when $p \leq 0.01$ using Statistica version 6.1 (Statsoft Inc., OK, USA) (*I*).

Pooled data sets of CQ or S plasma concentrations were used for the modeling purpose, using a mixed-effect modeling approach (NONMEM, version V, Globomax, Hanover, MD) (*IV*). In both models, the first-order conditional estimation with interaction option (FOCE INTERACTION) in NONMEM was used. Discriminations between hierarchical models were based on the objective function value (OFV) provided by NONMEM at a significance level of $p < 0.001$, equal to a decrease of 10.8 in the OFV, graphical analysis of residuals, and predictions in model diagnostics using Xpose, version 3.1 [Jonsson and Karlsson, 1999].

2.5 STATISTICAL METHODS

In the healthy volunteer study (*I*) data were presented as median values. All parameters were compared between treatment groups by the non-parametric Kruskal-Wallis test. To adjust for multiple PK comparisons, statistical significance was assumed only when $p \leq 0.01$. Statistical calculations were

performed using Statistica version 6.1 (Statsoft Inc., OK, USA). The Student's t-test was used to compare the volunteer characteristics.

In the clinical trial study (*II* and *III*) data capture was in Microsoft Excel spreadsheet, and preliminary analysis was done using SPSS version 10.0.05 (SPSS Inc. Chicago, IL). Further analysis was carried out using MINITAB version 14 (Minitab Inc. State College, Pennsylvania 2003). In papers *II – IV*, baseline characteristics were expressed as percentage for categorical variables, or as mean with standard deviation (SD) for continuous variables.

Treatment outcome, in *III*, was dichotomised into cure (Adequate Clinical and Parasitological Response) and failure (combining Early Treatment Failure, Late Clinical Failure, and Late Parasitological Failure) (WHO, 2003). The anthropometrics indices were calculated using the ANTHRO software (ANTHRO Version 1.02, CDC/WHO). The assessments of the anthropometrics were reported as Z-scores for the different sexes. For purposes of uniformity, the children were classified as stunted (ht-for-age Z-scores < -2), underweight (wt-for-age Z-scores < -2), or wasted (wt-for-ht Z-scores < -2). All children within ± 2 Z-scores were considered normal, as recommended in the International Reference Standard (IRS) (UNICEF, 2003). During analysis, these indices were also dichotomised into below -2 Z-scores and above -2 Z-scores. Logistic regressions were performed to assess associations between the dichotomised treatment outcome and various covariates. Due to dependencies among covariates, models with single covariates were mainly used to test associations, while to identify important covariates models with multiple covariates were used with backward conditional analyses. Regression of

individual PK parameters (CL, VC and AUC) against the covariates (fail/cure) was applied to assess the influence of sex, age, body weight, and height (**IV**). Categorical variables were compared using the Chi Square test, and continuous variables by the independent samples *t* test, and statistical significance assumed when $p \leq 0.05$ (**II – IV**). Residual plots were used to check distributional assumptions (**III – IV**).

2.6 ETHICAL CONSIDERATIONS AND CLEARANCE

The experiments in healthy volunteers and the clinical trial were performed in accordance with the Helsinki Declaration and the ICH recommendations. Informed consent from the participants was obtained by signature of the participant (**I&II**), or signified by a left thumbprint of the parent/caretaker of the children (**II**). No personal identifiers were included in the study reports. All participants/parents/caretakers were informed that they were free to withdraw from the studies at any time, and that the decisions would be respected. Ethical clearance was sought from the Higher Degrees and Research Ethics Committee at Makerere University and the Uganda National Council for Science and Technology who independently approved the study protocols. The Regional Ethical Review Board at Karolinska Institutet in Stockholm also approved the studies (**No.270/03** for the healthy volunteers study, and **No.2005/ 1251-31/2** for the clinical trial study).

3 RESULTS AND DISCUSSIONS

3.1 PARTICIPANT CHARACTERISTICS AND FOLLOW-UP

There were no significant differences in the age, body weight, liver function, creatinine levels and haemoglobin among the healthy volunteers in the four different groups before medications (*I*). In pharmacokinetic studies, wide variations in volunteers' anatomic or physiological functions, may affect drug disposition following their administration (Rowland and Tozer, 3rd Ed.) It is therefore important that volunteers selected are anatomically and physiologically as similar as possible. However, the emphasis for the characteristics of the children in the clinical trial study (*II*) was not based on those with similar anatomical or physiological characteristics, but rather it was based on the criteria for uncomplicated falciparum malaria within the specified age groups. Therefore, the possibility of variations in host characteristics were anticipated, hence the studies to determine the effects of these variations (anthropometrics) on pharmacokinetics and response to the drugs (*III*), from which a population model was developed (*IV*).

All participants in the pharmacokinetics study in healthy volunteers could be sampled on all occasions during the 21 days of study (*I*), while 164/183 (90%) of the children in the clinical trial study completed the 28 days of follow-up. That is a lost-to-follow-up of 10% (*II*). This was within the acceptable limits, where up to 15% lost-to-follow-up is taken as reasonable in clinical trials [Sullivan, 2004]. Of the children treated with CQ+SP, 83/86 (99%) were evaluated for the importance of drug levels and host factors in treatment outcomes (*III*), and from these, 498 sample concentrations were used as data points in modeling the population pharmacokinetics of CQ and S (*IV*).

3.2 BIOAVAILABILITY AND BIOEQUIVALENCE OF CQ, S AND P

Sulfadoxine was more rapidly absorbed ($k_a = 0.55 \text{ h}^{-1}$) given as Homapak than when given as Fansidar + CQ ($k_a = 0.27 \text{ h}^{-1}$, $p=0.004$). Its absorption was comparable to that in Fansidar alone ($k_a = 0.32 \text{ h}^{-1}$, $p=0.03$) (**Figure 2**). The median absorption time (t_{\max}) were 4h, 6h, and 10h for Homapak, Fansidar alone and Fansidar+CQ groups respectively. Other pharmacokinetic parameters (C_{\max} , AUC, $t_{1/2}$, CL and V_d) were not significantly different between the groups. No significant differences were observed for CQ in all the groups. The absorption of S given as Homapak was more rapid than as Fansidar. One possible explanation for this could be due to different pharmaceutical preparations of the tablets [Handbook of Pharmaceutical Excipients, 2nd Ed.]. Rapid dissolution of tablets forms in the gastro-intestinal tract may lead to more rapid systemic absorption [Clinical Pharmacokinetics: Concepts and Applications, 3rd Ed.]. A rapid absorption, as was seen for S in Homapak, is desired for an antimalarial formulation for rapid parasite clearance. Slow absorption may cause delayed parasite clearance and slower clinical response.

The relative bioavailability of CQ in Homapak was 0.97 (90%CI: 0.89 – 1.20), and that of S was 1.07 (90%CI: 0.94 – 1.12), thus showing bioequivalence for CQ and S when given as Homapak compared with GMP made CQ (Pharco) or S/P (Fansidar) [EMA, 2001]. In poor resource countries locally made generic drugs are often used [Brundtland GH, 2002; Quick JD et al, 2002], and therefore, it is important to establish that they are not of substandard quality or even faked [McGregor A, 1997; Rozendaal J, 2001; Taylor RB et al, 2001; Behrens RH et al, 2002].

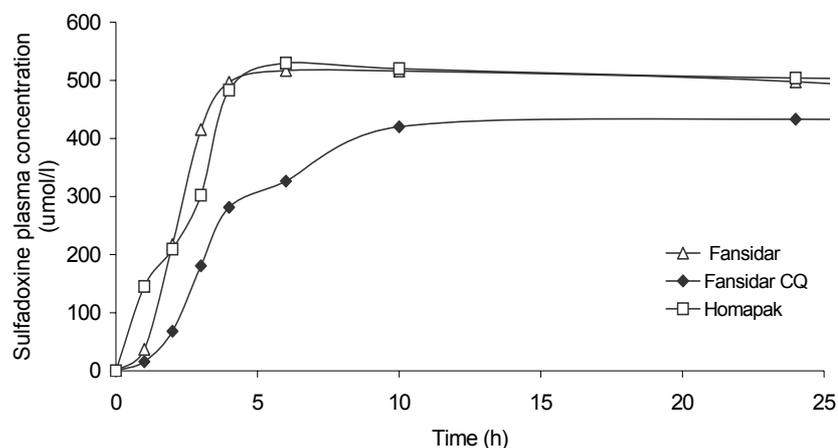


Figure 2: Absorption kinetics of Sulfadoxine in Homapak and GMP products.

Pyrimethamine plasma concentrations could only be obtained during the absorption and early elimination phase, but with no differences in the C_{max} , t_{max} and AUC_{0-24h} in the first 24 hours between the groups (*I*). Due to lack of samples left after analysis of CQ and S, bioequivalence could not be fully studied for P. However, there were no differences in absorption of P during the first 24 hours (**Figure 2**). Based on these findings, there is probably good bioavailability of P in Homapak. However, in the absence of complete pharmacokinetic data on pyrimethamine, it is not possible to unequivocally conclude that Homapak is bioequivalent to GMP made drugs.

3.3 INTERACTIONS AND TOLERABILITY OF THE DRUGS

There is always the potential for pharmacokinetic interactions when different drugs are co-administered [Stockley's Drug Interactions, 6th Ed]. Such

interactions may cause altered pharmacokinetic profile of any of the compounds in the combination, the consequence of which may lead to enhanced or inhibited pharmacodynamic effects during therapy with the possibility of adverse reactions [Ali HM, 1985; Grace JM et al, 1998; Rengelshausen J et al, 2004]. In this study there were no significant pharmacokinetic interaction between CQ, S and P (*I*). Furthermore, among the healthy volunteers, the drugs were generally well tolerated without any serious adverse reactions reported. This is an important finding, since the three compounds are used together as first line treatment of falciparum malaria in several resource poor nations (Giao PT, de Vries PJ, 2001; Maitland K et al, 2004).

In the clinical study (*II*) in children with uncomplicated falciparum malaria, the most common drug related complaint reported was abdominal pains. This was more common in children treated with the fixed-dose CQ+SP (22.2%) vs. AQ+SP (9.5%), $p=0.02$. The one case of severe anaemia that was observed 24 hours after the first dose of CQ+SP was probably caused by malaria complication. Other minor complains reported were general body weakness, loss of appetite, body itching, which were not common and occurred equally with both medications. No deaths occurred during the study. AQ medication was well tolerated in the children, although the study was not powered and designed to evaluate rare severe haematological and hepatic adverse reactions [Hatton CS et al, 1986; Neftel KA et al, 1986]. As mentioned earlier obtaining additional blood samples to do WBC analysis would put unnecessary burden on the children. However, despite this limitation, the absence of major serious adverse reactions in this study is in conformity with the findings from other studies [Adoke Yeka et al, 2005; Bakayita et al, 2005; Martensson et al, 2005; Mockenhaupt et

al, 2005; Sowunmi et al, 2005], where a combined a total of 2,479 children were treated with AQ for uncomplicated falciparum malaria, and no rare severe adverse reactions were reported. Thus, confirming that AQ may be used for therapeutic purposes, with minimal dangers of severe adverse reactions [Olliaro et al, 1996; Bloland, 2003].

3.4 EFFICACY OF AQ+SP AND FIXED-DOSE CQ+SP (II)

The clinical trial was conducted in 183 children (mean age 28.01 months, SD 14.66), with 99 in the fixed-dose CQ+SP (Homapak) arm and 84 in the AQ+SP arm. The fixed-dose CQ+SP formulation had inferior efficacy compared to AQ+SP combination in the treatment of uncomplicated malaria. The Primary outcome at day 14, by per protocol (PP) analysis (Table 6), showed clinical failure rates of 2.6% with AQ+SP, and 29.1% with the fixed-dose CQ+SP ($p<0.001$). Parasitological failure at day 28, after PCR adjustment for reinfection, was 13.1% with AQ+SP compared to 31.3% with fixed-dose CQ+SP ($p=0.003$). The mean fever clearance time (FCT) in the CQ+SP was 24 (SD 19) hours, while it was 18 (SD 15) hours in the AQ+SP group (NS). The parasite clearance time (PCT) in AQ+SP group was 2.8 days (SD 1.3) significantly shorter than the 3.7 days (SD 1.9) in the CQ+SP arm ($p<0.001$), such that by d3 the parasite burden was higher in the CQ+SP than in the AQ+SP groups (**Figure 3**). By d28, 18/86 (22.1%) children treated with CQ+SP were carrying gametocytes compared to 7/78 (9%) children given AQ+SP ($p=0.01$). The median change in Hb by d28 was an increase of 0.45g/dl in the AQ+SP arm, and 0.25g/dl in the CQ+SP arm (NS).

This study clearly demonstrates that the pre-packed fixed-dose formulation of CQ+SP (Homapak) has poor clinical and parasitological efficacy against uncomplicated falciparum malaria in Ugandan children compared to AQ+SP. Thus confirming the superiority of AQ+SP over that of CQ+SP combination found in other areas in the country [Gasasira et al, 2003; Bakyaita et al, 2005; Yeka et al, 2005]. At the time this study and the subsequent publication (*II*) a recommendation to replace CQ with AQ in combination with SP after a parasitological failure rate with AQ+SP was found below 25% was based on the WHO, 2003 recommendations. In 2006 the WHO revised the level of parasitological failure for effecting a drug change to PCR adjusted 10% parasitological failure by day 28 [WHO, 2006], a factor that may weaken the recommendation for CQ replacement with AQ in combination with SP. However, the efficacy study clearly indicates that it may be possible to use AQ in ACT combination, especially in low resource countries [Olliaro et al, 1996; Bloland, 2003]. AQ is more palatable than CQ [WHO, 1997], which improves compliance in children. Furthermore the documented good acceptability of Homapak by the community [Killian et al, 2003] would facilitate the introduction of a similar fixed-dose AQ+AS formulation [MoH-Uganda, 2006].

Despite the differences in the clinical and parasitological responses, there was no difference in the mean fever clearance time between the two arms. This could be explained by the fact that both AQ and CQ have anti-pyretic and anti-inflammatory effects [WHO, 1997]. The shorter mean parasite clearance time and the significant reduction in gametocyte rates with AQ+SP compared to CQ+SP are in agreement with earlier reports [Sowunmi and Fateye, 2003]. Furthermore, the rapid parasite clearance by AQ+SP combination may have

contributed to the suppression of gametocyte carriage, although other unknown mechanism may also be involved. On the other hand, the high gametocyte carriage in the CQ+SP treated children may facilitate the spread of resistant strains to the drugs [Tjitral et al, 2002]. The generally low mean Hb concentration at day 0 and day 28 that was observed is a common pattern in malaria endemic areas with intense transmission [Mockenhaupt et al, 2003].

Table 6: Day 14 therapeutic response by treatment groups (PCR-uncorrected)

Treatment	Clinical and parasitological outcomes						
	ACPR (%)	ETF (%)	LCF (%)	LPF (%)	Total Failures ^a (%)	Failure OR	95% CI (Sig)
AQ+SP n=78	97.4	0	0	2.6	2.6	1.0	
CQ+SP (Homapak) n=86	70.9	4.7	5.8	18.6	29.1	15.57	3.38 – 99.16 (<0.001)
Within CQ+SP (Homapak) comparison							
Full Strength (2-5years) n=55	81.8	1.8	1.8	14.5	18.2	1.0	
Half Strength (6-24months) n=31	51.6	9.7	12.9	25.8	48.4	4.22	1.52 – 11.27 (0.004)

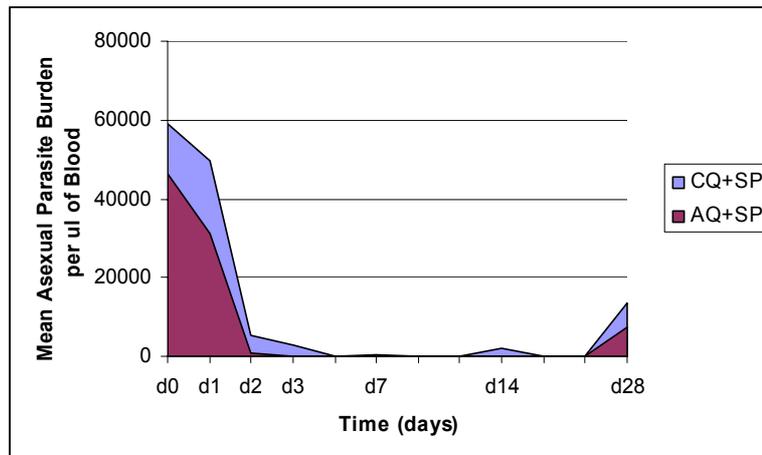


Figure 3: Parasite clearance with CQ+SP and AQ+SP

3.5 EFFICACY WITHIN THE CQ+SP SUBGROUPS (II)

Efficacy within the fixed-dose CQ+SP subgroups, showed day 14 failure rate of 18.2% with the full strength (FS) dose compared to 48.4% in the half strength (HS) dose ($p=0.002$) (Table 6). The parasitological failure with the FS dose was 15.8% but with corresponding higher rate of 52.4% in the HS dose group after PCR adjustment for reinfection ($p<0.001$). The PCT in the FS dose group was 3.0 days (SD 2.0) compared with the 4.7 days (SD 1.9) in the HS dose group), $p=0.001$ (II).

The above findings clearly show that the older children had better treatment outcomes compared to the younger age group. One possible explanation for the poor outcome among the younger children in the CQ+SP “half strength” subgroup could be the relationship between age and treatment response, where young age has been associated with poor treatment outcomes in malaria treatment [Dorsey et al, 2002; Hamer et al, 2003]. Age is only one variable. It becomes necessary to explore other possible reasons for the poor efficacy.

Importantly however, was the fact that few children in the study developed ETF, an outcome that is associated with high risks for complications and mortality. No deaths were reported in the present study, although it was in the low dose CQ+SP group that most cases of ETF were recorded, and where the two cases of severe complications occurred.

In high transmission areas, parasite reappearance during therapy could be due to new infections or due to recrudescence, which may lead to wrong classification of the outcomes [WHO, 2003]. Genotyping techniques offer the best methods for characterising parasite response [Basco and Ringwald, 2000; Farnert et al, 1997]. In this study, of the commonly analysed genes, the MSP-2 gene that was analysed has been shown to be quite robust in discriminating between reinfection and recrudescence [Cattamanchi et al, 2003; Basco and Ringwald, 2001]. However, to further reduce the risk for wrong classification it would be useful to determine additional genes (MSP-1 and GLURP), but this was not possible in the present study.

3.6 MALNUTRITION AND IMPORTANCE IN TREATMENT WITH FIXED-DOSE CQ+SP (III)

Malnutrition was common among the children, with stunting as the most frequent nutritional/growth status abnormality, constituting 27.7% of the study population (n=83), followed by underweight 19.3%, with only 9.6% found wasted. Ten percent of children had concurrent stunting and underweight. The proportion of stunting in the HS group was nearly 4 times that in the FS group (51.6% vs. 13.5%), which resulted in children in the HS dose group being significantly more stunted (mean ht-for-age Z-scores = -1.55) than those in the FS dose group (ht-

for-age Z-scores = -0.53 , $p=0.005$) (Table 7). No significant differences were found in the proportions of wt-for-age (22.6% vs. 17.3%) and wt-for-ht (6.5% vs. 11.5%) between the HS and FS dose groups respectively. However, significantly higher odds for cure was found among the non-stunted in the FS compared to the HS dose groups (36/45 vs. 5/15, $p=0.003$) (///). By performing a backward conditional logistic regression in the whole group, ht-for-age Z-scores ($p=0.046$) was kept in the model as the significant explanatory covariate for treatment outcomes together with the d1 S and d3 CQ concentrations (section 3.7)

Table 7: Anthropometric indices between fixed-dose groups

Anthropometrics ^a	Half strength dose group n=31		Full strength dose group n=52		Mean difference (95%CI of difference)	p value
	Mean	SD	Mean	SD		
Ht-for-Age Z-score (stunting)	-1.55	1.55	-0.53	1.55	1.02 (0.32 – 1.73)	0.005
Wt-for-Age Z-scores (underweight)	-1.14	1.05	-0.94	1.16	0.20 (-0.31 – 0.71)	0.435
Wt-for-Ht Z-score (wasting)	-0.21	1.27	-0.70	1.27	-0.49 (-1.06 – 0.09)	0.093

^aThese indices are not expected to differ within and between the age groups, the value for each index is expected to be = 0 in normal children.

The proportions of stunting, underweight, and wasting found in this study are similar to the proportions that have been found in other surveys within the region in the under fives (Kikafunda et al, 1998; Nyakeriga et al, 2004; Matee et al, 1997) Within the region, stunted children form nearly one-third of the under-fives [Kikafunda et al, 1998; Nyakeriga et al, 2004]. But while malnutrition and malaria may be associated with poor treatment outcomes [Caulfield et al, 2004; Hung et al, 2005; Kikafunda et al, 1998], it is impossible to ascribe the impact of malaria

disease to malnutrition in this study, especially where the nutritional status of the study population prior to the malaria episodes could not be established (///). Furthermore, the lack of locally derived anthropometrics reference data may also be an important limitation as the evaluation nutritional status was conducted using the WHO reference standard that may not be wholly applicable to the population in the study [UNICEF, 2003; WHO, 1995]. However, the important question that these findings bring to the fore is whether stunting really is important for the outcome of *P.falciparum* malaria. This needs to be clarified in future larger studies.

3.7 GIVEN DOSE, ATTAINED CONCENTRATIONS AND THEIR IMPORTANCE IN TREATMENT WITH FIXED-DOSE CQ+SP

Despite the efforts to ensure that only children with no history of antimalarial drug intake were recruited prior to the study treatment, of the 83 children 37.2% had CQ and S alone or concurrently in the pre-treatment blood samples. The median pre-treatment concentration was 132 nmol/l (range 49 – 1270 nmol/l) for CQ and 79 µmol/l (range 15 – 164 µmol/l) for S, with no difference between the HS and FS dose groups. No difference was found in d14 cure rates between the children with and those without pre-treatment drug concentrations (///). This, in a way showed how reliance on antimalarial drug medication history is a limitation to this kind of study [Ogbuokiri, 1990; Terlouw et al, 2003; Quashie et al, 2005]. Quashie et al (2005) had shown that pre-dose levels of CQ influenced the treatment outcomes, but in this study no such association was found, as the levels were probably too low to contribute any significant additional effects.

The mean given doses of CQ and S, and the mean d1 S concentration were significantly higher in the FS compared with the HS group (Table 8), and overall higher mean daily concentrations of CQ and S in the FS dose group compared to the HS dose group was found (**Figure 4**). However, using logistic regressions analysis for the individual variables in the whole study population, the given doses of CQ ($p=0.007$) and S ($p=0.007$), and the d1 S concentration ($p=0.001$) were individually significantly associated with cure or failure. And nearly all children with day 1 S concentrations above the population mean ($319 \mu\text{mol/l}$) were cured (**III**).

Table 8: Given dose and drug concentrations in the whole group and between HS and FS dose groups

Treatment	Whole Group N=83	HS n=31	FS n=52	T-test FS-HS difference p values (95%CI)
CQ Given dose (mg/kg) Mean (SD)	31 (7)	24 (4)	34 (6)	<0.001 (7.6 – 12.6)
Day 3 Conc. (nmol/l) Mean (SD)	1922 (1092)	1681 (1157)	2095 (1026)	0.149 (-153.3 – 981.2)
S Given dose (mg/kg) Mean (SD)	34 (8)	27 (4)	38 (7)	<0.001 (8.4 – 14.0)
Day 1 Conc. ($\mu\text{mol/l}$) Mean (SD)	319 (121)	258 (84)	354 (126)	0.001 (40.4 – 150.9)

The working assumption with the fixed-dose CQ+SP formulation was that children receiving the full strength dose group were on average twice the weight of those in the half strength dose group (NMCP-Uganda, 2002). The two dose groups would in theory receive approximately equal amounts (in mg/kg body weight) of the drugs. But the actual doses (mg/kg) received during treatment were higher, with higher concentrations and better cure rates in the FS dose group. The importance of given dose has previously been reported in studies from Guinea-Bissau (Kofoed et al, 2002) and from Rwanda (Sexton et al, 1988), when double doses (50mg/kg) or the usual doses (25mg/kg) of CQ were given to

children with uncomplicated falciparum malaria, and the children treated with double doses had higher drug concentrations and better cure rates.

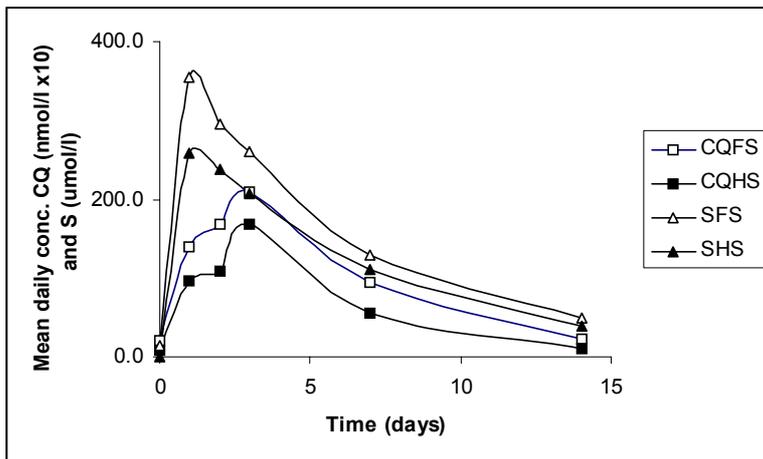


Figure 4: The mean concentration-time curves of CQ and S in dose groups^a.

^aCQHS and SHS stand for chloroquine (CQ) and Sulfadoxine (S) concentrations in the half strength (HS) dose group, while CQFS and SFS stand for CQ and S concentrations in the full strength (FS) dose group. Peak trough concentrations observed on day 1 (median) and day 3 (median) for S and CQ respectively.

In the determination of the explanatory covariates, multiple logistic regressions were performed for the whole study population by modelling treatment outcome with all covariates (S given dose, day 3 CQ concentration, day 1 S concentration, sex, wt-for-age Z-scores, wt-for-ht Z-scores, ht-for-age Z-scores, Hb day 0, parasitaemia day 0, and temperature day 0). Due to collinearity, some covariates (MAC, age, weight, height and CQ given) were excluded from this analysis. The d1 S concentration was found to be the only significant explanatory variable ($p=0.045$). Finally, backward conditional logistic regression was performed, after starting with the full model as above, and only d1 S concentration ($p=0.004$), d3

CQ concentration ($p=0.037$) besides ht-for-age Z-scores (section 3.6) were kept in the model as the significant explanatory covariates for treatment outcomes. The findings in this analysis show that drug concentrations were important for the treatment outcomes. Hellgren et al (1989) had similarly shown that higher concentrations were associated with better parasitological response in Tanzanian children given CQ for asymptomatic falciparum malaria.

It has been shown that nearly all children who failed had concentrations below the population mean with an almost two-fold difference in the given dose. The implication of this finding has a bearing on the new fixed-dose schedules recently proposed for the administration of co-formulated artemether-lumefantrine combination (Coartem) in Uganda. The schedule indicates that children between 4 months to 3 years or weight range of 5kg to 14 kg, will all be given the same fixed dose twice daily for three days for uncomplicated malaria (NMCP-Uganda, 2006). There will therefore be a three-fold difference in the given dose (mg/kg) between the age/weight extremes. This might, in accordance with the present study, cause treatment failure in those given the lowest dose in mg/kg body weight. It can be suggested that in order to accommodate variations in the anthropometrics, narrower graded age-dose intervals should be considered.

3.8 POPULATION PHARMACOKINETICS OF CQ AND S IN CHILDREN WITH UNCOMPLICATED FALCIPRAUM MALARIA (IV)

3.8.1 Population pharmacokinetic parameter estimates in the whole group

Using sparse data from individual children with uncomplicated malaria treated with fixed-dose CQ+SP, population pharmacokinetic parameter estimates of the apparent volume of distribution (V_p) and total clearance (CL) for both CQ and S

were best described by 2-compartmental models with a first-order absorption rate constant (k_a) (Table 9). For S, the addition of absorption lag time (approximately 30 minutes) improved the fit of the model significantly.

Table 9. Table 2: Population pharmacokinetic parameter estimates for SDx and CQ in 83 children aged 6 – 60 months

Model Parameters ^a	SDx estimate (RSE%)	IIV (RSE%)	CQ Estimate (RSE%)	IIV (RSE%)
V_c (L/kg)	-	-	31.8 (20)	0.57 (67)
V_p (L/kg)	1.6 (16)	-	230 (7.8)	-
CL (L/h/kg)	0.023 (5.3)	0.33 (35)	2.84 (5.4)	0.3 (35)
CLD ₂ (L/h/kg)	0.13 (15)	-	3.29 (11)	-
k_a (h ⁻¹)	0.30 (24)	-	0.14 (13)	-
Lag-time (h)	0.29 (14)	-	Not estimated	-

V_c : central volume of distribution, V_p : volume of peripheral compartment, **CL**: total clearance, **CLD₂**: inter-compartmental clearance values, k_a : absorption rate constant; **RSE**: relative standard error in percent; **IIV**: inter-individual variability

This study has shown that bi-compartmental models best described the pharmacokinetics of CQ and S in children treated for uncomplicated falciparum malaria. For CQ, this is in conformity with findings previously reported in children with malaria [Walker et al, 1983, Adelusi et al, 1982], as well as in the healthy volunteers study in this thesis (*I*) also as reported by other investigators in adults [Frisk-Holmberg et al, 1979; Gustafsson et al, 1983].

Although in the healthy volunteers (*I*) and in a recent study (Barnes et al, 2006) S disposition was reported as one-compartmental, a two-compartmental pharmacokinetics of S in the present study is in agreement with earlier reports in [Weidekamm et al, 1982; Edstein, 1987; Sarikabhuti et al, 1988]. This contrast in

the compartmental disposition of S may be due to differences in the sampling periods or in the pharmacokinetic analysis approach (individual vs. population modelling), especially where no modelling was applied in the earlier studies.

3.8.2 Summary pharmacokinetic parameters in the younger and older age groups

Post hoc (summary pharmacokinetic) parameter estimates in the individual age categories showed significant differences for S, C_{max} (130 $\mu\text{g}/\text{mL}$ vs. 171 $\mu\text{g}/\text{mL}$, $p < 0.0005$) and AUC_{0-336} (12,500 $\mu\text{g}\cdot\text{h}/\text{mL}$ vs. 16,900 $\mu\text{g}\cdot\text{h}/\text{mL}$, $p < 0.0005$) in the younger vs. older age groups respectively. And similarly for CQ, the C_{max} (0.79 $\mu\text{g}/\text{mL}$ vs. 1.43 $\mu\text{g}/\text{mL}$) and AUC_{0-336} (77.5 $\mu\text{g}\cdot\text{h}/\text{mL}$ vs. 140 $\mu\text{g}\cdot\text{h}/\text{mL}$, $p < 0.0005$) respectively in the younger vs. older children (**IV**). These differences may be as a direct effect of the different dosages given, with influence from the individual host nutritional status variability earlier presented (**III**). However, in a recent study, where it was presumed that all the children had been given equivalent of 25mg/kg and 1.25mg/kg of S and P respectively, differences in AUC were still observed in the different age categories [Barnes et al, 2006]. Comparison of the summary PK characteristics in this study appeared to show more similarities with those in study **I**, as opposed to the Barnes et al, 2006 study (**Table 10**). The C_{max} in both the HS and the FS groups in this study appear to be 2 – 3 times higher than values in the Barnes et al study with similar doses of S. The contrasts may be ascribed to methodological differences, thus making it impossible to compare the results (different sampling times, and the analytical methods employed). Perhaps the only findings where there appears to be some agreement in this study (**IV**) with the Barnes et al study is that the values of C_{max} and AUC tend to be higher in the older than in the younger age groups.

In the present study we have applied a population approach [Beal and Sheiner, 1982] to evaluate the pharmacokinetics of CQ and S in the fixed-dose CQ+SP combination [Whiting et al, 1986; Aarons, 1991; Kauffman and Kearns, 1992]. During the PK analysis, a number of samples were discarded as a result of procedural difficulties from the frequent sampling in children as described earlier, which also lead to failure to collect adequate samples for the analysis of P levels. Thus limiting discussions to CQ and S only, and in this case S levels have been used as surrogate for P levels in the combination (*IV*). While only CQ and S levels were analysed, discarding samples visa vice the sample size usually has the effect of lowering of the power of the study. Since population approach was applied to the study, participants could give any number of samples. In this case the number of samples varied (between one to eight) while the number participants remained the same. Furthermore the samples were pooled before analysis such that the discarded samples would not significantly affect the results.

3.8.3 Exposure (AUC_{0-336}) and response

This study demonstrates that the drug exposures (AUC_{0-336h}) for CQ and S individually were significant predictors for cure. Combined exposures of the two drugs were also significant predictors for cure, thus showing that the exposure by 336 h may be important for therapy with CQ and S. Earlier exposures (AUC_{0-24h} and AUC_{0-72h}), however, did not appear to significantly predict cure within the given dosages of CQ and S. Non-response was often associated with low AUC_{0-336} values for CQ and S that were more commonly seen in the lower age group (*IV*). Barnes et al (2006) recently found that poor response was associated with lower AUC_{0-7d} in the 2 –5 year age group compared to older children (>12 years).

Another study also reported no significant difference in $AUC_{0-\infty}$ of S between those who cured and those who failed treatment [Dzinjalama et al, 2005]. This divergent finding could suggest that AUC is perhaps not a very suitable surrogate marker for treatment outcomes. A larger study is necessary to ascertain the relevant exposure (AUC) for cure with either CQ or S, and to determine which time-point for AUC exposure is important for prediction of cure.

Table 10: Comparison of S summary pharmacokinetic parameters in children with uncomplicated malaria and in healthy volunteers

S summary PK parameters	Children Uncomplicated malaria (<i>IV</i>)		Adult Volunteers (Homapak)	Barnes et al, 2006 Uncomplicated malaria	
	6 – 24 m	2 – 5 yrs	20-30 yrs (<i>I</i>) ^a	2 – 5 yrs	> 12 yrs
Samples	FP/blood	FP/blood	Plasma	FP/blood	FP/blood
Mean S dose (mg/kg)	27	38	27	38.5	27.3
Compartmental Model Used	Two	Two	One	One	One
C_{max} ($\mu\text{g/mL}$)	130	171	164	57.9 ^b	63.2 ^b
$AUC_{0-\infty}$ ($\mu\text{g}^*\text{d/mL}$)	N/A	N/A	2,053	460 ^c	816 ^c
AUC_{0-336} ($\mu\text{g}^*\text{d/mL}$)	520 ^d	704 ^d	N/A	N/A	N/A
T_{max} (h)	5.7	7.0	4	N/A	N/A

^a μg data converted from original equivalents of μmol or mmol in *I*

^b C_{max} reported by Barnes et al 2006 were estimates by day 1, the actual peak concentration during the absorption phase not captured.

^cThe lack of concentration data during absorption phase may be responsible for the rather low AUC values in comparison to findings in this study

^dValues equivalent for $\mu\text{g}^*\text{h/mL}$ in *IV*

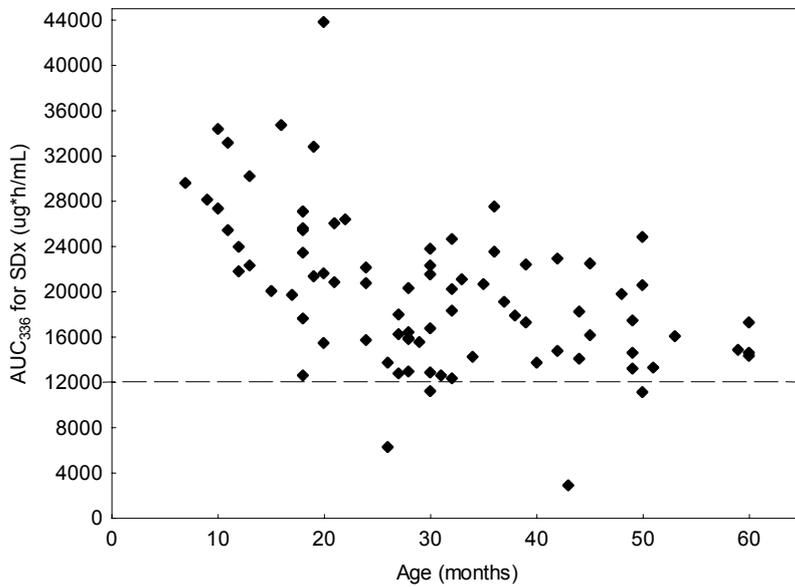
FP/blood = whole blood on filter paper

3.8.4 Proposed modification of dose regimen in children 6 months to 5 years

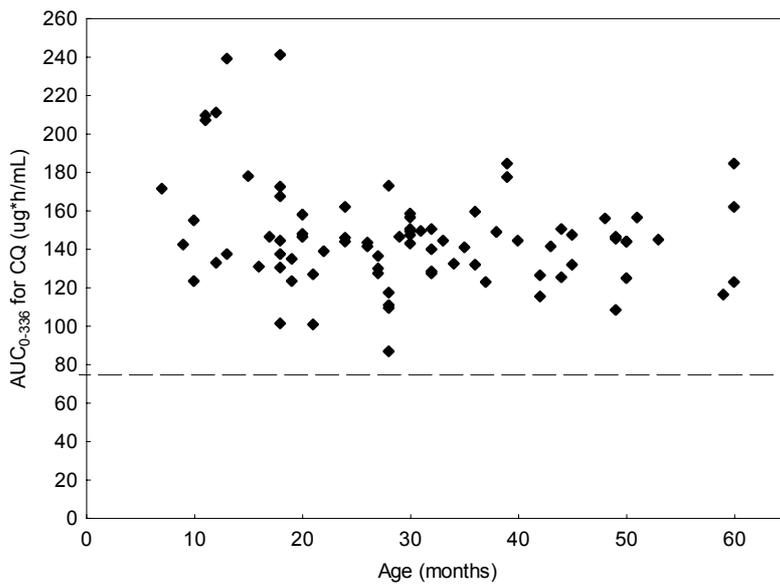
In this study, compartmental pharmacokinetic models were developed for CQ and S in children ranging from 6 to 60 months in age. The population pharmacokinetic model for CQ did not imply any correlation between body weight and age with the model parameters, while the S modeling results indicate an effect of body weight on the apparent central volume of distribution for the compound. The lack of covariate influence on CQ pharmacokinetics is an interesting finding and implies the current empirical age-based dosing to be irrational in this age range. Furthermore, these results suggest a weight-based dosing of S to be more appropriate than the current dosing based on age in children older than 6 months. Thus, the current practice to administer 50% lower doses at ages 6 - 24 months therefore seems sub-optimal. In a model where if all children were given the higher dose, only four children in the whole group would achieve S AUC_{0-336h} values below the suggested cut-off value (12,000 $\mu\text{g}\cdot\text{h}/\text{mL}$) for treatment response, and no child would have AUC_{0-336h} values below the corresponding cut-off value (76 $\mu\text{g}\cdot\text{h}/\text{mL}$) for CQ (**Figure 5**).

In proposing a dose modification, from a safety perspective it is important to avoid exceedingly high concentrations during the initial phase of dose intake, however, a strong relationship between high drug exposure of S and CQ and cure was found. And predicted C_{max} levels after from the proposed dose modification showed that only three children relatively higher C_{max} predictions, but not very different from some individuals given the current dosage regimen (**IV**). However, for countries where these drugs are still relatively efficacious,

these findings show that, response with CQ+SP combination can be improved by giving the higher dosage irrespective of body weight. Higher dosages of CQ (50mg/kg) [Kofoed et al, 2002; Sexton et al, 1988], and for SP (1000mg/50mg) [Barnes et al, 2206] have been previously proposed for improved outcomes in uncomplicated malaria. Therefore, in this study, a recommendation for children between 6 months to 24 months to be administered the higher dose regimen given to the older children can be done within safe C_{max} limits to avoid any possible adverse effects (*IV*).



a) Sulfadoxine



b) Chloroquine

Figure 5: Predicted AUC_{0-336} vs. age (a) for S and (b) for CQ if the higher Homapak dose had been administered to all children 6 months to 5 years. Broken line depicts optimal cut-off value for AUC_{0-336} to predict treatment response.

3.9 CONCLUSION

The fixed-dose chloroquine plus sulfadoxine/pyrimethamine formulation, locally known as Homapak, is of good quality with respect to CQ and S, but with the data on P lacking, it cannot unequivocally be said to be bioequivalent to the GMP products. There is no clinically relevant interaction between chloroquine, sulfadoxine and pyrimethamine. The current dose regimen of half-strength Homapak to the younger children results in poor response. But treatment with AQ plus SP has superior efficacy against CQ plus SP combination. AQ could therefore be used as an alternative to CQ in combination with SP. Patient factor such as stunting and the given doses affect the pharmacokinetic parameters thus influencing the treatment outcomes with fixed-dose CQ+SP. A proposed dose modification to give the full-strength dose to all children in the age range 6 months to 5 years would improve treatment outcome with Homapak.

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