DETERMINANTS OF RESPONSES TO ANTIMALARIAL DRUGS IN CHILDREN WITH UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA

BY

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ABSTRACT

Drug resistance is a challenge to malaria control efforts and several factors including parasite genetics, host factors and pharmacokinetics may contribute to the selection of drug resistant *Plasmodium falciparum*. Understanding the role of these factors in patient response to antimalarial drugs is therefore essential in the management of malaria. The aim of the study was to determine the factors contributing to delay in malaria Parasite Clearance (PC) in children and evaluating the effects of pharmacokinetic variability on treatment outcome.

Children (n=2,752), aged 6 months -12 years, with *falciparum* malaria, were enrolled over a period of eight years and treated with standard doses of Chloroquine (CQ), Sulphadoxine-Pyrimethamine (SP) or Amodiaquine (AQ) given alone or in combination with artemisinin. Each patient was followed up for at least 14 days. Age, axillary temperature, parasite density and gametocytaemia were assessed for their potential association with delay in PC and treatment outcomes. Filter-paper blood samples were collected from some of the children (n=148) before treatment and on days 1-7, 14, 28 and 35 after treatment for determination of CQ and sulphadoxine concentrations. In another subset of patients (n=7), treated with amodiaquine, blood and saliva samples were collected over 35 days. High performance liquid chromatographic techniques were used to determine concentrations of sulphadoxine in whole blood as well as AQ and Desethyl amodiaquine (DEAQ) in saliva. Mean maximum drug concentration (Cmax), half-life ($t_{1/2}$) and area under concentration-time curve ($AUC_{0\text{-}28d}$) were assessed for their association and predictive value for treatment outcomes. Data were analyzed using descriptive statistics, ANOVA, Chi-square, Students' t-test, Kruskal-Wallis and multiple regressions at $p = 0.05$.

Age ≤ 2 years (Adjusted odds ratio [AOR] = 2.13), presence of fever (AOR = 1.33) and parasitaemia > 50,000/µl (AOR = 2.21) at enrolment were independent risk

factors for delay in PC, while a body temperature >38^{O}C and parasitaemia >20,000/µl were predictors a day after treatment regardless of the drug used. Day 3 concentration ≤ 1750ng/ml and $AUC_{0\text{-}28d}$ ≤ 950ng/ml.h were associated with chloroquine treatment failure. In a multivariate analysis, a terminal elimination $t_{½}$ ≤ 220h (AOR = 0.28) and $AUC_{0\text{-}28d}$ ≤ 950ng/ml.h (AOR = 4.12) were identified as independent pharmacokinetic predictors of chloroquine treatment failure. Age stratified analysis showed that SDX concentrations were significantly higher in children > 5years compared to children <5years: Cmax; 295 vs 125µg/ml, $AUC_{0\text{-}28d}$; 1562 vs 812µg/ml.d. In patients who received AQ, there was a rapid conversion of AQ to DEAQ, which was detectable in plasma and saliva within 40 minutes of administration. The mean day 7 concentration of DEAQ was significantly higher in plasma than in saliva (247.8 vs 125.1ng/ml). The $t_{1/2}$ of DEAQ were similar in plasma (167.25±43.4h) and saliva (146.12±17.2h). The decline phases of DEAQ in saliva concentration-time curves were approximately similar to that in plasma.

Delay in parasite clearance is specific and related to drug resistance. In addition pharmacokinetic variability of sulphadoxine in children has potential impact on dosage regimen and treatment outcome.

Keywords: **Uncomplicated malaria, Pharmacokinetics, Antimalarials, *Plasmodium falciparum*.**

Word Count = 499

DEDICATION

To the children who participated in the studies used for this thesis.

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CERTIFICATION

I certify that this work was carried out by Mr SIJUADE, Abayomi Olusola of the Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan.

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Glossary of Abbreviations

ACPR	Adequate clinical response
ADP	Adenosine diphosphate
AOR	Adjusted odd ratio
ATP	Adenosine triphosphate
AL	Artemether plus lumefantrine
AMQ	Mefloquine plus artesunate
ANOVA	Analysis of variance
AOR	Adjusted odd ratio
AQ	Amodiaquine
AQAS	Artesunate plus amodiaquine
AQPS	Amodiaquine plus sulphadoxine-pyrimethamine
AQSP	amodiaquine plus sulfalene-pyrimethamine
AS	Artesunate
$AUC_{0\text{-}28d}$	Area under concentration time curve from 0 to 28 day
CI	Confidence interval
Cl	Clearance
C_{max}	maximum concentration
COT	Co-trimozaxole
CQ	Chloroquine
CQCP	chloroquine plus chlorpheniramine
CQKET	Chloroquine plus ketotifen
CQPS	Chloroquine plus sulphadoxine-pyrimethamine
d	Day
DCQ	Desethyl chloroquine

DEAQ	Desethyl amodiaquine
df	degree of freedom
DHA	Dyhydro artemisinin
DHPS	Dihydropteroate synthase
DHFR	Dihyrdofolate reductase
DNA	Deoxyribonucleic acid
dUMP	deoxyuridine monophosphate
dUTP	deoxyuridine triphosphate
ETF	Early treatment failure
F	female
Fig.	Figure
gcm^{-3}	Gramm per centimetre cube
gdm^{-3}	Gramm per decimetre cube
GM	Geometric mean
GTP	Glutathione reductase
h	hour
Hb	Haemoglobin
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
IPT	Intermittent preventive treatment
IS	Internal standard
ITNs	Insecticide treated nets
Kg	Kilograms
L	Litre
LTF	Late treatment failure

M	Molarity
MDR	Multi drug resistance
ml	millilitre
min	minutes
MQ	Mefloquine
mV	Millivolt
n	Sample size
$NaH_2PO_4.2H_20$	Sodium dihydrogen phosphate
NaOH	Sodium hydroxide
ng/ml	Nano gram per Millilitre
^oC	Degree Celsius
OR	Odds ratio
pABA	Paraaminobenzoic acid
PCT	Parasite clearance time
PYR	Pyrimethamine alone
PPV	Papaverine
RBM	Roll Back Malaria
SP	Sulphadoxine-pyrimethamine
SPPB	Sulphadoxine-pyrimethamine plus probenecid
SD	Standard deviation
SDX	sulphadoxine alone
$t_{1/2}$	Half-life
t_{max}	Time at maximum concentration
V/V/V	Volume/volume/volume
VS	Versus

WHO	World Health Organization
y	Year
$\chi 2$	Chi square

CHAPTER ONE

INTRODUCTION

Significance of the project

Among several tropical diseases that affect humans, malaria poses special control problems due to increasing population at risk from the disease. In 2008, there were approximately 232 million malaria cases and 841,000 malaria deaths, with close to 90% of these occurring in sub-Sahara Africa (WHO 2009). Nigeria is the largest population at risk in Africa. Being a serious disease that mostly affects children under the age of 5 year, it is responsible for 25% of infant mortality and 30% of all childhood deaths in Nigeria (FMOH 2004). Globally, it causes 85% of death in children under 5years of age (Global Malaria, 2008). It is a significant cause of morbidity accounting for about 65% of all clinic attendance in the country (FMH 2005). Nigeria is the first among the top-five countries for malaria death (Global Malaria, 2008). In the absence of an effective antimalarial vaccine, chemotherapy remains the mainstay for control of the disease. However, the malaria parasite has developed resistance to nearly all the classes of antimalarials including the promising artemisinin derivatives (Dondorp *et al*., 2009). This could be as a result of widespread and indiscriminate use of antimalarials, which places a strong selective pressure on malaria parasites to develop high levels of resistance (Olumese 2005; WHO 2006).

The reports of antimalarial drug resistance in disease endemic countries, especially Africa (Sowunmi & Salako 1992; Falade *et al*., 1997; Wang *et al*., 1997; Sibley 2001; Sowunmi *et al*., 2004; Talisuna *et al*., 2004; Pitmang *et al*., 2005) had

made the WHO to recommend Artemisinin based combination therapy (ACTs) for the treatment of acute uncomplicated malaria. Between 2002 and 2005 many African countries including Nigeria adopted the use of ACTs (FMH 2004). Unfortunately, ACTs are expensive for poor people living in disease endemic countries and require longer dosage regimen which pose challenges in their use. In addition, the emergence and spread of resistance to these front-line antimalarials have necessitated the need to continually field-monitor parasite susceptibility to conventional antimalarial drugs such as chloroquine (CQ) and sulphadoxine-pyrimethamine (SDX) in order to revisit them for the treatment of acute uncomplicated malaria (Nkoma *et al*., 2007; Dondorp *et al*., 2009). For instance, in Malawi chloroquine regained it efficacy for treatment of malaria 12 years after it was withdrawn from use, suggesting that CQ might once again be considered for treatment of malaria (Laufer *et al*., 2006).

Indeed, it is very important to determine factors that can be observed prior to treatment that can lead to treatment failure especially in children. In this perspective, it would clearly help if all, or, at least, most children at risk of drug treatment failure could be identified soon after they become ill. This would allow the planning of alternative, more effective treatment strategies and thus the reduction of malaria-attributable morbidity and mortality and slowing of the spread of drug resistance.

A fundamental component of the strategy for the control of malaria disease is based on prompt and effective treatment. In vivo tests are much more direct measurement of treatment efficacy in a target population. The assessment of in-vivo therapeutic efficacy involves clinical and parasitological outcomes of treatment over a certain period following the start of treatment in order to check for the reappearance of parasites in the blood. A significant proportion of treatment failures do not appear

until after day 28 in areas of low or high transmission (WHO 2006). However, it is not often clear if patients have adequate drug blood levels at the time of recrudescence of infection and this can be determined by monitoring of blood concentration after treatment which can also be used to predict treatment outcome (White *et al.*, 2008). Pharmacokinetics can also play a major role in the development of drug resistant malaria. It has been demonstrated that pharmacokinetics of drugs can be altered during long time use (Bousquet, 1970). Chloroquine and sulphadoxine-pyrimethamine have been common drugs used in the treatment of malaria. The long-time use of CQ and SP might have caused changes in their kinetic disposition which may result in selective drug pressure (Gardella *et al.*, 2008; Hodel *et al*., 2010). Thus, the current study was designed to re-assess drug resistance level and determine whether drug treatment failures observed during a standard in-vivo test are related to parasite factors or whether they are the consequence of poor metabolism of CQ and SP.

Although, chromatographic techniques have been developed to allow accurate and sensitive determination of the level of most antimalarial drugs in blood (Karbwang *et al*., 1987; Walker *et al*., 1983; Walker & Ademowo 1996; Babalola *et al*., 2003; Minzi *et al*., 2005; Dua *et al*., 2007) these techniques are quite expensive, time consuming and require the collection and use of venous blood. The sampling techniques require expertise, are invasive and sampling may not be easy in children during field studies. These techniques may not be very practicable in analytical laboratories in low resource areas where malaria is endemic. Efforts in this project were devoted to the optimization and development of simple and cost effective chromatographic techniques for measurement of CQ and sulphadoxine in micro blood samples collected on filter paper. The newly developed method is

sensitive and requires less sample which are easy to transport. This new method of analysis will provide a unique opportunity to facilitate SDX blood level determination in patients and can also be employed for therapeutic drug monitoring during Intermittent Preventive Treatment for malaria control in pregnant (IPTp) women or children (IPTc) using sulphadoxine-pyrimethamine.

Besides, the determination of antimalarial drug levels has been estimated in general from whole blood, plasma or red cell (White 1992; Salako and Sowunmi, 1992; Gbotosho *et al*., 2009; Obua *et al*., 2008). However, antimalarials have also been estimated in saliva, for example, quinine (Salako and Sowunmi, 1992). Although, there is much difficulty in measuring artemisinin drugs in biological samples, it is often easier to measure their partner drugs, e.g. amodiaquine or sulphadoxine plus pyrimethamine (Gitau *et al*., 2004; Gbotosho *et al*., 2009; Obua *et al*., 2008). An ideal medium from which antimalarial drugs should be measured should be non-invasive with respect to sample collection; saliva is one of such medium (Salako and Sowunmi 1992; Wilson *et al*., 1993).

Despite increasing drug treatment failure, there are no clear guidelines, at least in Nigeria, about the time to change antimalarial drug treatment if parasites do not clear quickly from peripheral blood following treatment of uncomplicated acute infections in African children. Effort in this thesis was devoted to understanding the clinical factors that can lead to antimalarial treatment failure and the pharmacokinetic basis of treatment failure in children with acute uncomplicated *P. falciparum* malaria in an area of intense malaria transmission in Nigeria.

Objectives of the research:

1. To determine the relationship between delay in parasite clearance and

antimalarial treatment failure in children with falciparum malaria.

2. To determine the pharmacokinetic risk factors and the effects of pharmacokinetic variability on treatment outcome in children with acute uncomplicated falciparum malaria treated with chloroquine.
3. To develop and evaluate a simple, cost effective and sensitive method for quantification of sulphadoxine in small capillary samples of whole blood spotted on filter paper.
4. To assess the field applicability of the developed method for sulphadoxine: to study the pharmacokinetic disposition of sulphadoxine and evaluate the effect of pharmacokinetic variability on therapeutic efficacy in children.
5. To evaluate the use of saliva for therapeutic drug monitoring in patients treated with amodiaquine-artesunate combination.

CHAPTER TWO

LTERATURE REVIEW

2.0 Malaria the disease

Malaria, the most prevalent and most pernicious disease of human, remains up till now a major endemic parasitic disease and a leading cause of morbidity and mortality especially in sub-Sahara Africa (WHO 2006). In the world as a whole, malaria has been a major disease of mankind for thousands of years. The global morbidity and mortality due to malaria have not significantly changed over the past 50 years (Greenwood, 2004). World Health Organization (WHO) reported an increase in malaria clinical cases from 273 millions in 1998 to 515 millions cases in 2002 (Snow *et al.*, 2005).

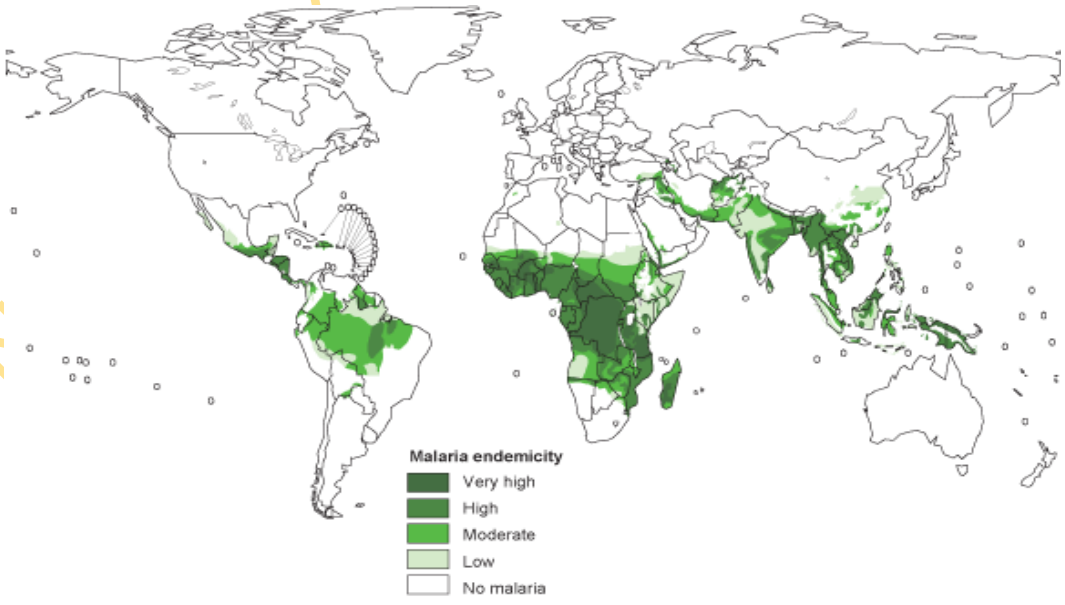


Figure 2.1: Global distribution of Malaria in 2003 (*http://rbm.who.int/wmr2005/html/map1.htm*, Adopted from World Malaria Report 2005).

Malaria is a hematoprotozoan parasitic infection transmitted by species of anopheles mosquitoes (White 2004). It is currently endemic in 90 countries of the world of which a substantial population is from Africa, South of Sahara (Figure 2.1) (Kondrachie & Trigg, 1997; Olumese, 2005). Malaria accounts for one in five of all childhood deaths in Africa (Kager, 2002; RBM, 2006). The disease causes over 1 million deaths, 75% of which occur in African children < 5 years. In Nigeria, malaria accounts for 63% of the diseases reported in health care centres and prevalence of malaria among pregnant women is 48% (FMOH, 2004). Almost US$ 3.5 million was reported by the Nigerian government for funding of malaria control in 2003, with an additional US$ 2.3 million from other sources (RBM, 2005).

Non-immune travellers visiting malaria endemic areas are at risk of malaria infection because of lack of immunity which develops after repeated infection (Hviid, 2005; Stevenson & Zavala, 2006). Pregnant women especially primigravidae are also at risk of malaria infection (Whitty *et al*., 2005; WHO, 2003; Adam *et al.*, 2005) due to reduced immunity in pregnancy and the presence of the plancenta which offer a highly supportive growth environment for the parasite (Serghides *et al.*, 2001). Malaria in pregnancy leads to maternal anaemia (Miaffo *et al*., 2004), low birth weight and premature delivery, which are associated with an increased risk of neonatal death and impaired cognitive development. Economically, the impact of malaria is alarming and contributes to individual, community and country poverty through lost labour days and expenses incurred for treatment and prevention. The disease impairs physical and mental development in children (Kihara *et al*., 2006).

2.1 Life Cycle of Plasmodium

There are various species of the genus plasmodium responsible for the disease

in man which includes *P. falciparum, P. vivax, P. malariae* and *P. ovale* (White, 2004; Crutcher & Hoffman, 2001). The most important of these is *P. falciparum* because it can rapidly cause fatal infections and it is responsible for the majority of malaria related deaths. The life cycle of the malaria parasite is split between a vertebrate host and an insect vector (Figure 2.2). The extreme rarity of *P. vivax* in West Africa is apparently due to prevalence of the duffy negative trait in West Africans. This is an inherited red cell phenotype that lacks the receptor for invasion of the human red cell by the merozoites of *P. vivax*.

When a mosquito infected with the malaria parasite bites human, developmental stages of the parasite called sporozoites is injected into the human's bloodstream (A). The sporozoites then travel to the liver (B). Each sporozoite undergoes asexual reproduction, in which its nucleus splits to form two new cells, called merozoites. Merozoites enter the bloodstream and infect red blood cells (C). In red blood cells, merozoites grow and divide to produce more merozoites, eventually causing the red blood cells to rupture. Some of the newly released merozoites infect other red blood cells (D). Some merozoites develop into sexual stages known as male and female gametocytes (E). When another mosquito bites the infected human, ingesting the gametocytes (F). The gametocytes mature in the mosquito's stomach and undergo sexual reproduction, uniting to form a zygote (G). The zygote multiplies to form sporozoites, which travel to the mosquito's salivary glands. If this mosquito bites another human, the cycle begins again (H).

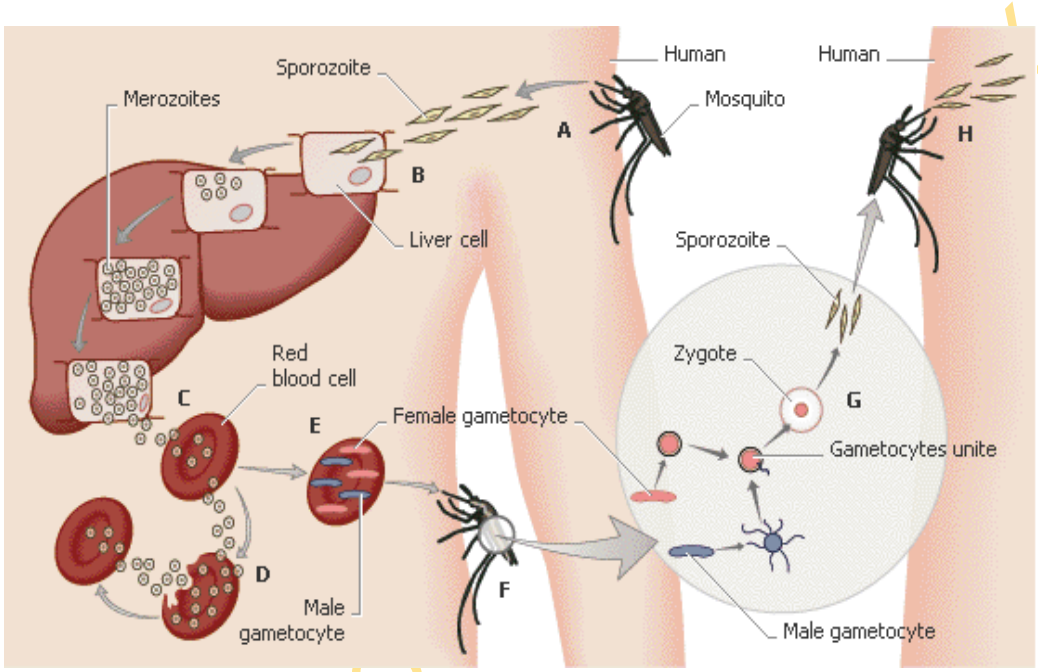


Figure 2.2: *Plasmodium spp.* Life Cycle

(http://encarta.msn.com/media_461541582/Life_Cycle_of_the_Malaria_Parasite.html)

2.2 Malaria Control Methods

Global Malaria Control and Disease Management

Malaria control efforts are currently focused on two major interventions which include targeting the mosquitoes that transmit malaria by the use of insecticide-treated bed net (ITNs) and early diagnosis and treatment of malaria (EDTM). These are two of the pillars of global malaria control campaign (WHO 2000; Olumese, 2005; Barat, 2006; Killeen, 2007). Prevention of malaria encompasses a variety of measures that may protect against infection or against the development of the disease in infected individuals. Measures that protect against infection are directed towards the mosquito vector preventing the transmission of gametocyte. These include personal (individual or household) protection measures e.g. protective clothing, repellents, bednets or community/population protection measure e.g. use of insecticides or environmental management to control transmission. Intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) in pregnant women is the primary prevention strategy that relies on the use of medication (White, 2005; Yartey, 2006; Schellenberg *et al*., 2006; Vallely *et al*., 2007) for control of malaria among pregnant women.

Several partnerships have been set-up by international organizations for effective control of infectious diseases especially malaria. These organizations include Malaria Control and Evaluation Partnership in Africa (MACEPA) and Roll Back Malaria (RBM). MACEPA's mission is to demonstrate that scaling up malaria prevention and control under national leadership saves lives, reduces illness, and increases economic opportunity (Macepa, 2007). The Roll Back Malaria partnership is an initiative to improve malaria control in the context of health sector reform. It

was initiated in 1998 through a joint partnership of WHO, UNICEF, UNDP and the World Bank. However this joint partnership has now embarked on a Global Malaria Control Programme. This new programmes focused more on reducing the morbidity and mortality associated with malaria. Accordingly, the objectives of the global malaria control strategy were prioritized as follows:

- Provision of early diagnosis and prompt treatment for the disease;
- Planning and implementation of selective and sustainable preventive measures including vector control
- Early detection of and response to malaria epidemics,
- Improved prevention and treatment of malaria in pregnant women.
- Strengthening of local research capacities to promote regular assessment of countries' malaria situations, in particular the ecological, social and economic determinants of the disease.

2.3: Chemotherapy of malaria

Chemotherapy remains one of the important practicable tool to control falciparum malaria in sub- Sahara Africa where > 90% of the world's burden of malaria mortality and morbidity occurs (Sibley, 2001). Chemotherapy in malaria as in other infectious diseases is based on preventing the growth or survival of infecting agents, by means of drugs without damage to the host. Effective treatment of falciparum malaria depends on a rapid reduction and clearance of parasitaemia. The malaria parasite is however developing resistance rapidly to most of these drugs.

2.3.1: Classification of antimalarial drugs

There are many antimalarial drugs with specific effect on various stages of the malaria parasite lifecycle that have been developed and are currently in clinical use. The stages of development of malaria parasite show varying degree of susceptibility to antimalarial drugs. These antimalarial drugs can be categorized according to chemical classes or stage of parasite against which they are most effective (Table 2.1).

Table 2.1: Classification of anti-malaria drugs

Class	Blood schizontocide	Tissue schizontocide
4-Aminoquinolines	Chloroquine	
	Amodiaquine	
Arylaminoalcohols	Quinine	
	Quinidine	
	Mefloquine	
Phenanthrene-methanol	Halofanthrine	
8-Aminoquinoline		Primaquine
Artemisinin and derivatives	Dihydroartemisinin	
	Artemether	
	Artesunate	
Antimetabolites	Proguanil	Proguanil
	Pyrimethamine	
	Sufadoxine	
	Sulfalene	
	Dapsone	
Antibiotics	Tetracycline	Tetracycline
	Doxycycline	Doxycycline
	Minocycline	Minocycline
WHO recommended combination therapy options for Africa		
Artemether plus lumefantrine		
Artesunate plus amodiaquine		
Artesunate plus sulphadoxine-pyrimethamine		
Dihydroartemisinin plus piperaquine		

2.3.2: Pharmacology of antimalarial drugs

Quinine

Quinine is a quinoline antimalarial (Fig. 2.3). It has been the drug of choice for the management of severe malaria in most areas of the world (WHO, 2005). Krishna *et al* (2001) reported that malaria parasites still remain sensitive to quinine in Africa despite resistance to commonly used quinolines although, in some part of South East Asia decrease sensitivity has been detected (RBM 2001). Recently, it was strongly suggested that parenteral artesunate should replace quinine as the treatment of choice for severe falciparum malaria worldwide. Artesunate was well tolerated, with no serious drug-related adverse effects with significant reductions in parasite clearance time (Roshental., 2008; Dondorp *et al.*, 2010). Quinoline antimalarials are known to have similar mechanism of action in malaria chemotherapy (Slater, 1993; Francis *et al.*, 1997). They are known to inhibit digestion of haemoglobin by the parasite and thus reduce the supply of amino acids necessary for parasite viability. Salako *et al*. (1989) reported a significantly lower clearance and longer half-life in children with kwashiorkor than normal children. Administration of quinine plus nevirapine results in significant decreases of AUC, C_{max} and $t_{1/2}$ in healthy volunteers (Soyinka *et al*., 2009). Adverse reactions are common with quinine therapy, but severe life-threatening toxicity is rare. A characteristic symptom complex known as cinchonism is caused by quinine (Powell & McNamara, 1966). These consist of tinnitus, high tone deafness (Roche *et al*., 1990), nausea, uneasiness, malaise, and blurred vision. Hypoglycemia is a more commonly encountered problem with quinine treatment. Quinine clearance is increased by

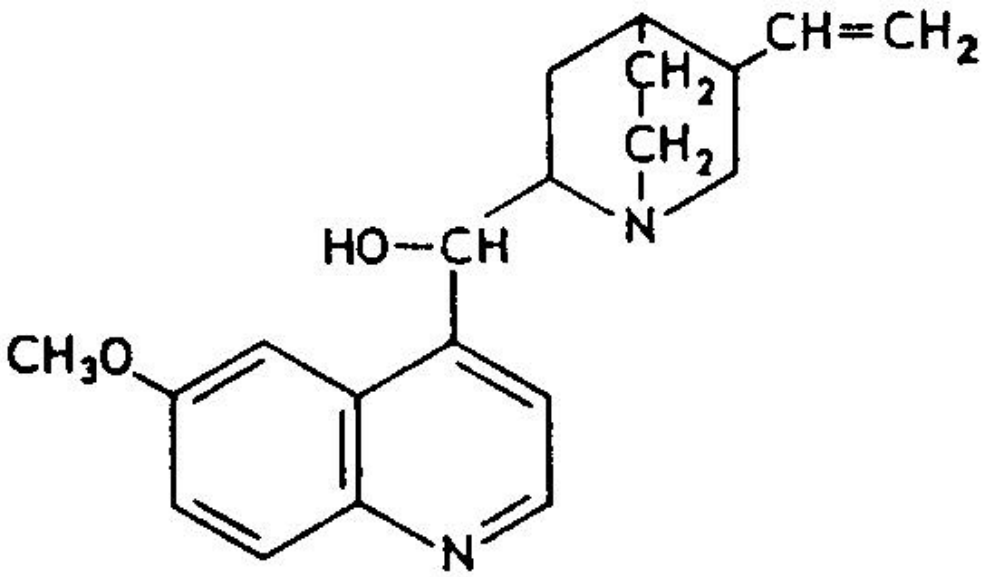


Figure 2.3: Chemical structure of Quinine

phenobarbitone, rifampicin (Pukrittayakamee *et al.*, 2003) and smoking, but reduced by cimetidine. The pharmacokinetic parameters of quinine are reported to be significantly altered during malaria and this alteration is in proportion to the severity of the infection. Malaria infection causes a reduction in volume of distribution and clearance leading to a prolongation of elimination half-life as well as elevated plasma drug concentrations (Babalola *et al*., 1998).

Mefloquine

Mefloquine is a quinoline-methanol compound, which is structurally similar to quinine (Fig. 2.4). It was developed by the Walter Reed Army Institute of Research (WRAIR), to combat emerging strains of drug resistant *P. falciparum* (Bryskier *et al*., 1988). Structurally, it consists of 2–α and piperidyl and

trifluoromethyl group in position 2 of the quinoline nucleus. Mefloquine is effective against all malaria species including multidrug resistant *P. falciparum*. Initially mefloquine against chloroquine resistant and sensitive strains of *P. falciparum* has been well reported (Doberstyn *et al*., 1997; Sowunmi *et al*., 1990; 1992). However significant resistance has developed in South East Asia (WHO 2000a). Oduola *et al*. (1987) reported a reduction in the in-vitro sensitivity of isolates of *P. falciparum* from West Africa to mefloquine even before the drug was introduced into the region, suggesting that parasite with innate resistance to mefloquine may be present in the West Africa sub region. The structure-activity relation is based on N-O of 2- α piperidyl group (Tracy *et al*., 1996).

The actual mechanism of action of mefloquine is still unknown however, it is thought to inhibit heme polymerase. Mefloquine forms toxic complexes with free heme causing damage to parasite membrane and interacts with other plasmodia components (Mockenhaupt, 1995). Mefloquine is metabolized by CYP3A4. An increase in plasma concentration was reported when mefloquine was co-adminisatered with ketoconazole, a CYP3A4 inhibitor (Ridtitid *et al.,* 2005). Rifampicin which induces CYP3A4 enzyme reduced plasma concentration of mefloquine when coadministered (Ridtitid *et al*., 2000). Mefloquine is generally well tolerated, although nausea, abdominal discomfort, dizziness, vomiting and diarrhea are the frequent adverse reactions associated with it (Phillips-Howard *et al*., 1995; Ter Kuile *et al.*, 1995; Sowunmi *et al*, 1990). There is no established biochemical basis behind neurotoxicity of mefloquine but it is found to disrupt neuronal calcium homeostasis and induce an endoplasmic reticulum stress response at physiologically relevant concentrations effect that may contribute at least in part of the neurotoxicity (Geoffrey *et al*., 2003). It also results in severe neuropsychiatry

reactions such as disorientation, seizures, encephalopathy, hallucinations and sleep disturbances (nightmares). Recently, mefloquine has been reported to induce pneumonitis (Soentjens *et al*., 2006).

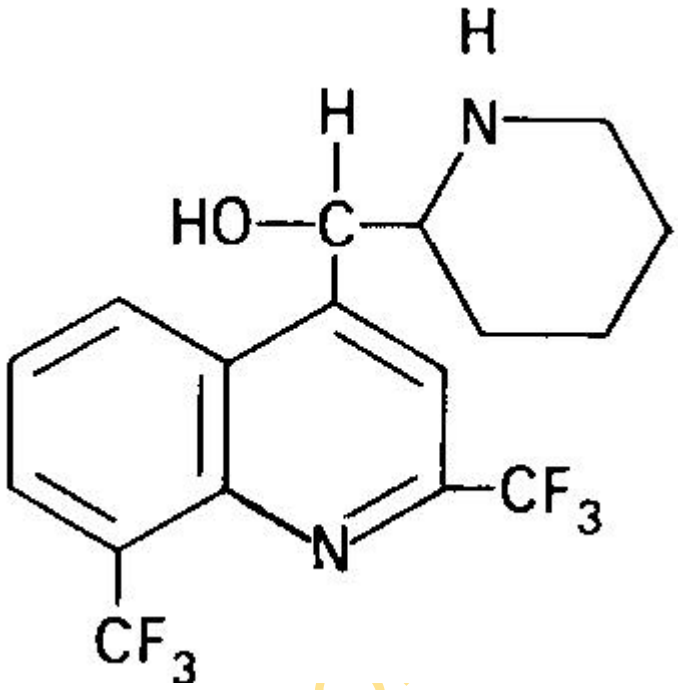


Figure 2.4: Chemical structure of Mefloquine

Halofantrine

Halofantrine is a 9-phenanthrene-methanol antimalarial agent (Fig. 2.5). It is a schizonticidal drug that probably acts by formation of a complex with Ferriprotoporphyrin IX toxic to the parasite. Inhibition of proton pump at the host-parasite interface has also been hypothesised as an alternate mode of action (Watkins *et al.,* 1988; Barriso & Goa 1992). It is active against all human malarial parasites. Minor and reversible event including nausea, diarrhea, vomiting and abdominal discomfort occur but are usually self-limiting. Pruritus has been reported in 13% of Nigerian taking the drug (Sowunmi *et al.*, 1989). It has no adverse effect on central nervous system and is better tolerated than mefloquine (TerKuile *et al*., 1993). Halofantrine prolongs Q-Tc interval (Dion *et al*., 2001; Darrel *et al*., 2001) and should not be administered to patients taking drugs known to prolong the QT interval (i.e. Quinine, tricyclic antidepressant, Quinidine) or to those who have received mefloquine within few days (White, 1996; Touze *et al*., 1997). Oral bioavailability is increased up to six-fold if halofantrine is taken with fatty meal (Shanks *et al*., 1992; Tracy *et al*., 1996).

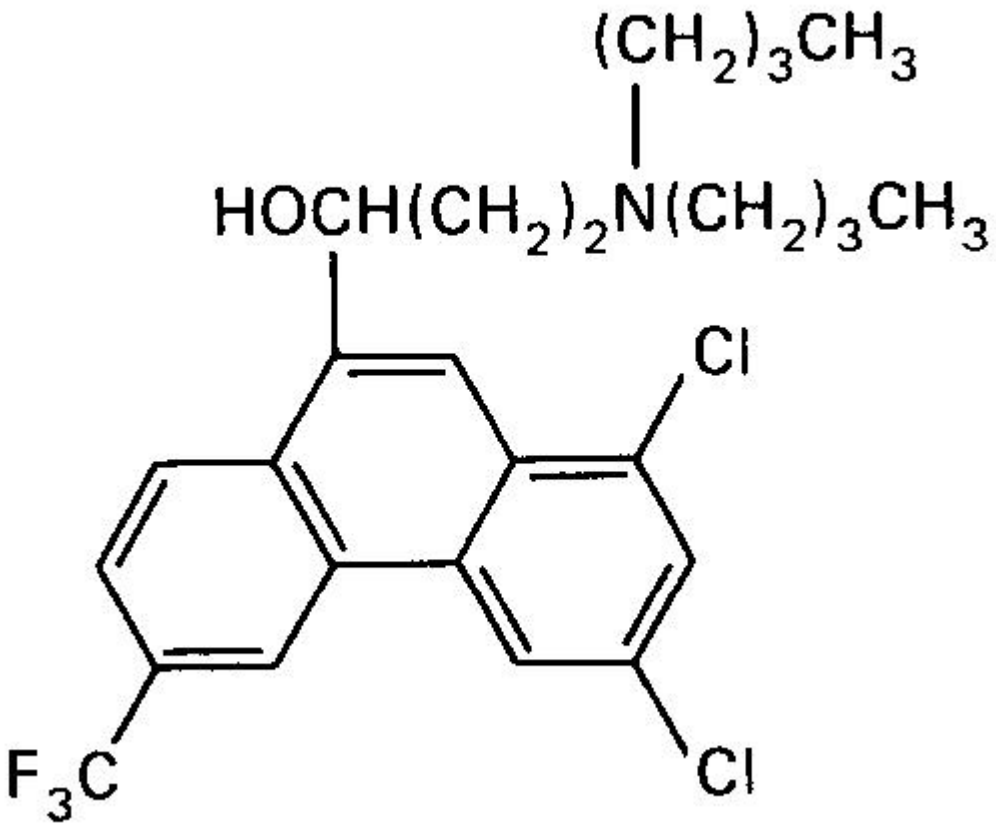
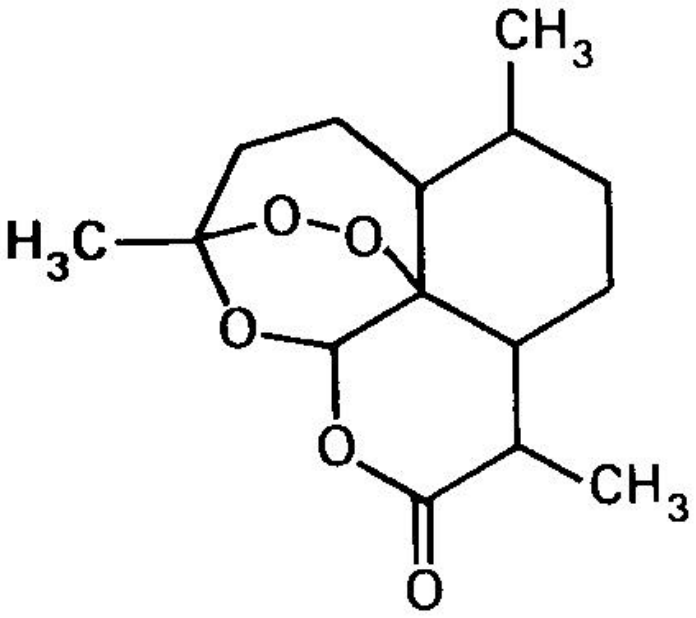


Figure 2.5: Chemical structure of Halofantrine

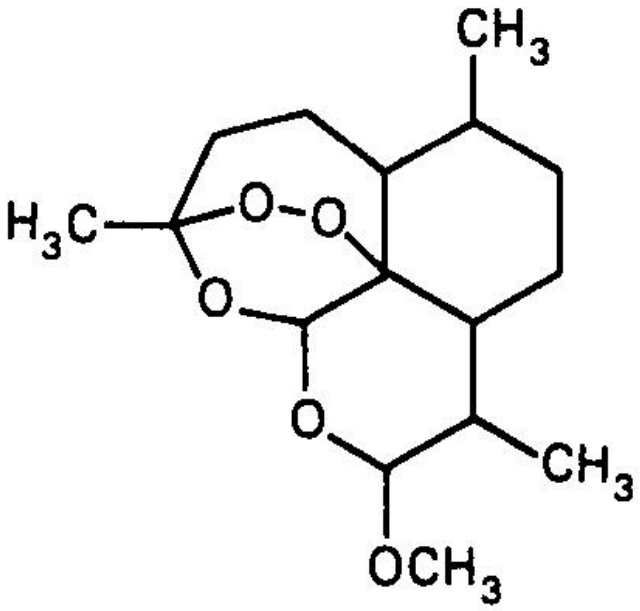
Artemisinin and its derivatives

The family of trioxane compound derived from the plant *artemisia annua* (qinghaosu) and a series of synthetic derivatives are among the most potent antimalarial compounds known (Fig. 2.6). The trioxane structure contains a peroxide group essential for its antimalarial activity (Klayman, 1985; Woerdenbarg *et al.*, 1994). These naturally occurring sesquiterpene lactones are structurally unrelated to the quinoline antimalarial drugs. Artemisinin derivatives include: Artemisinin, deoxyartemisinin, dihydroartemisinin (DHA), artesunate, artemisinic acid, artemisitene, arteether and artemether respectively. Three compounds have been extensively evaluated: the parent compound artemisinin, the water soluble artesunate and the oil soluble ether artemether.

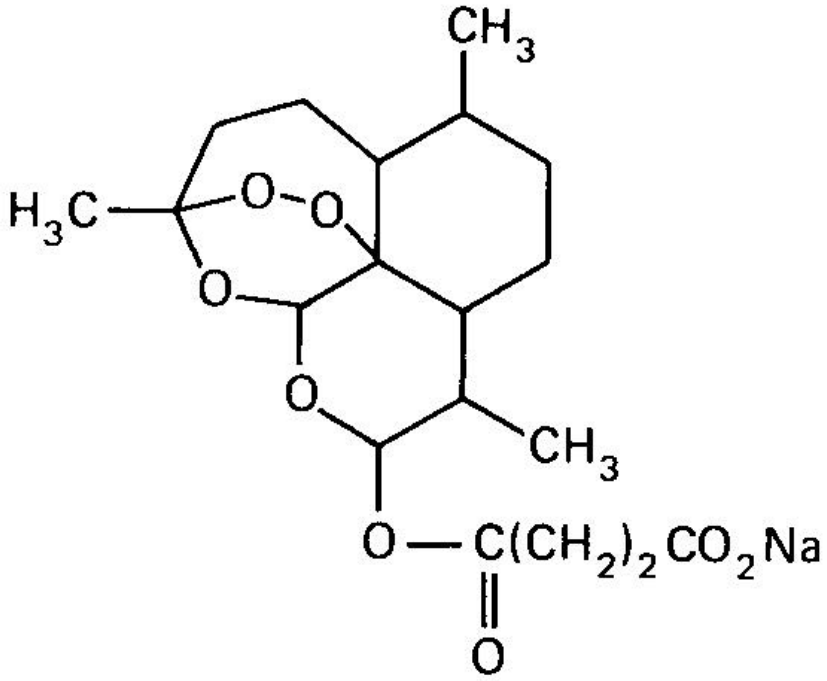
Artemisinins act very rapidly reducing parasitemia by a factor of 10^4 with each cycle (Baird *et al*., 2005; Baird, 2005; Wilairatana *et al*., 2002). Unfortunately, there are reports of reduced susceptibility of the artemisininsin the Thai/Cambodian border (Jambou *et al*., 2005; Dandorp *et al.,* 2009). Artemisinin can be given orally and intramuscularly. Artemisinin, but not other derivatives (Karunajeewa *et al*., 2004), shows considerable autoinduction of its own metabolism so that blood concentrations after several days of dosing are considerably lower than would be predicted from the initial dose (Ashton *et al*., 1998). Oral artesunate and artemether, but not artemisinin, are hydrolyzed rapidly back to the common metabolite DHA, which is intrinsically more active as an antimalarial agent (Li *et al*., 2007). Oral artesunate may be considered mainly as a prodrug for DHA, as the metabolite is the main contributor to overall antimalarial activity (Newton *et al*., 2000).



Artemisinin



Artemether



Artesunate

Figure 2.6: Chemical structure of Artemisinin derivatives

2.4: Review of studies on Pharmacokinetic of chloroquine, sulphadoxine-pyrimethamine and amodiaquine.

Chloroquine:

Chloroquine, is 7-chloro-4-(4-diethyl amino-1-methylbutyl-amino) quinoline, and a tertiary amine (Figure 2.7). It is the most important of the 4-aminoquinoline compounds for the treatment of malaria. The 4-aminoquinoline nucleus has a chlorine atom attached to 7th carbon atom of the nucleus. The chlorine atom at 7th position of the quinoline nucleus greatly confers the antimalarial activity on the molecules (Goodman & Gillman 1996).

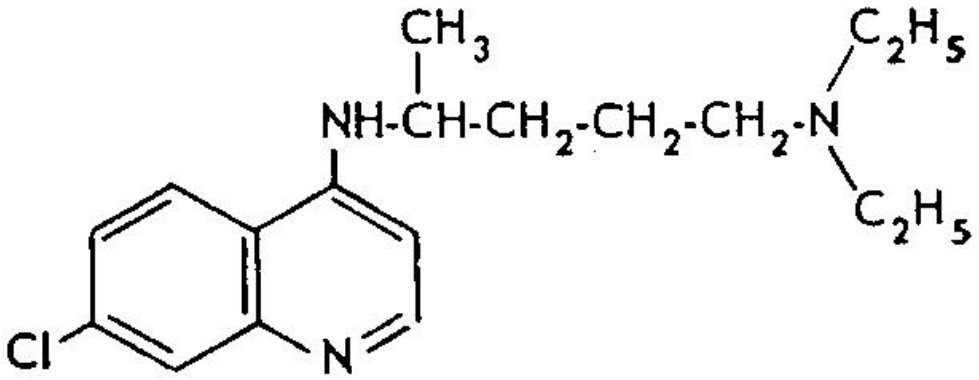


Figure 2.7: Chemical structure of Chloroquine

(i) Mode of action: Chloroquine is a weak base but its accumulation in the parasite lysosome is 1000-fold greater than predicted on the basis of a weak base effect (Rang *et al.*, 1997). Chloroquine acts on the intraerythrocytic *P. falciparum* stages that are responsible for the clinical manifestation of the disease. These stages feed on the erythrocyte haemoglobin, in acidic vacuoles (lysosomes). Chloroquine inhibits digestion of haemoglobin by the parasite and thus reduces the supply of amino acids necessary for parasite viability (Figure 2.4); it is also said to cause fragmentation of the parasite RNA and intercalate DNA. The toxic haem is polymerized into insoluble non-toxic haemozoin (Sarchez *et al*., 1997). *Plasmodial* heme polymerase catalyses this polymerization reaction and it is inhibited by chloroquine and other quinolines (Slater, 1993; Francis *et al.*, 1997). A higher parasite burden is associated with increased risk of failure of treatment with CQ and mefloquine (Sowunmi *et al*., 2004 & 2005).

Pharmacokinetics of chloroquine

***(i) Absorption*:** Chloroquine is rapidly absorbed and widely distributed after oral administration in healthy adults (Gustafsson *et al*., 1983) and children with uncomplicated malaria (Adelusi *et al.*, 1982). In adults with moderately severe malaria, bioavailability relative to parenteral treatment was 70% compared with 75% in healthy subjects (Gustafson, 1983). In children with uncomplicated malaria given an initial oral treatment dose of 10mg base/kg, peak plasma concentration of approximately 250µg/L was reached in 2h (Adelusi *et al.*, 1982). Absorption is very

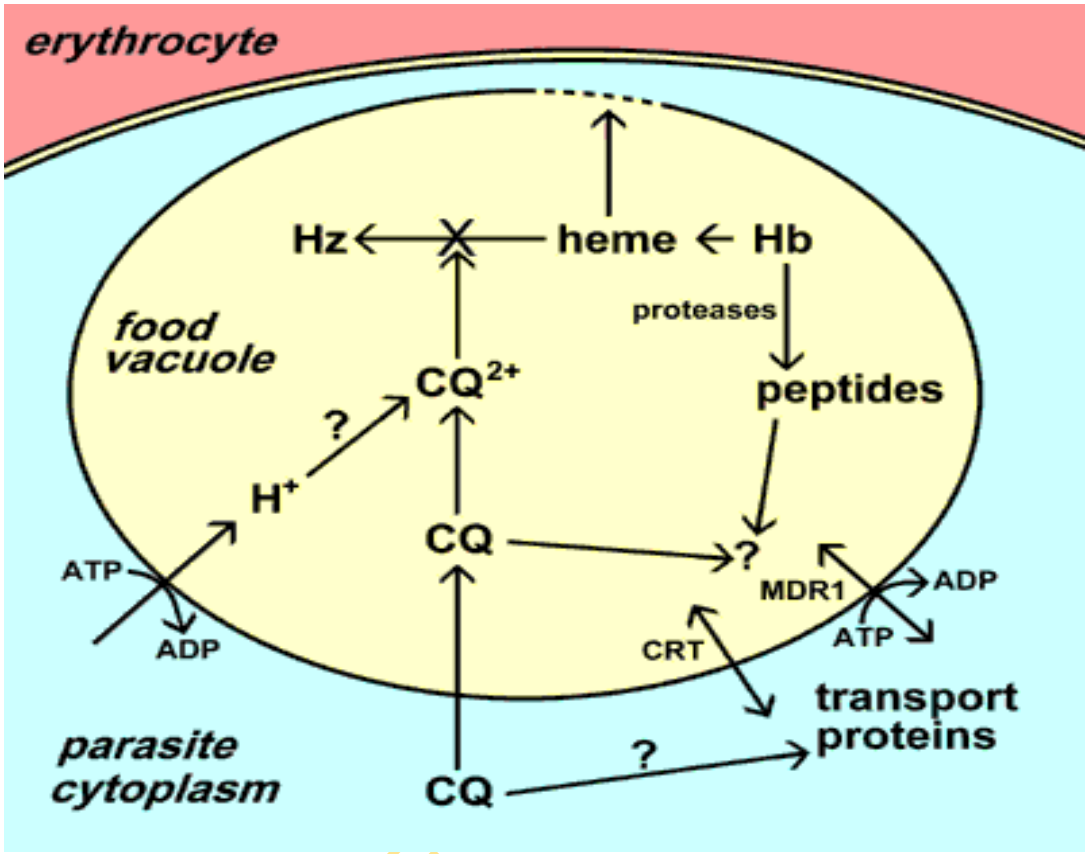


Figure 2.8 **Mode of action of Chloroquine**

rapid and relatively complete even in very severe infections (White *et al.*, 1987b, 1988). The absorption and elimination of chloroquine in children appears to be similar to that in adults (WHO 2000).

(ii) Distribution: Chloroquine is widely distributed throughout the body after absorption. It is extensively bound to tissues particularly liver, spleen, kidney, lungs, connective tissues and tissues containing melanin such as retina resulting in enormous apparent volume of distribution (Walker *et al.*, 1983). The pharmacokinetic properties of chloroquine are complex. The total apparent volume of distribution is enormous (100-1000 L/kg) because of extensive tissue binding (Frisk-Holmberg *et al*., 1984) whereas the volume of central compartment is several orders of magnitude smaller (0.18 ±0.13L/kg) (Looareesuwan *et al.*, 1986). The process of distribution from this central compartment determines the blood concentration profile during the treatment of malaria. Chloroquine concentration in red blood cells is approximately 3-5 times higher than in plasma, and there is considerable concentration in granulocytes and platelets (Nosal *et al*., 1988). Whole blood concentration is 6-10 times higher than plasma concentration (Gustafsson *et al*., 1983) and further in parasitized red blood cells (Adelusi *et al*., 1982; Ajayi *et al*., 1988). Chloroquine concentration in cerebrospinal fluid are very low, with a mean value of 2.7% of corresponding whole blood concentration (White, 1988b) and slightly concentrated in breast milk with area under the concentration time curve milk/plasma ratio of 2.0 - 4.3 (Edstein *et al*., 1986). Chloroquine in saliva has a long elimination half-life (7-20d) and there is a good correlation between the AUC values derived from saliva and plasma (Onyeji and Ogunbona 1996; Ogunbona *et al*., 1986).

(iii) Elimination: Chloroquine is eliminated slowly such that the drug and it metabolites can be detected in plasma for 21 - 60 days after single dose of 5mg/kg depending on the sensitivity of the assay method. It is 51% cleared unchanged by the kidney (Gustafsson *et al*., 1983; Frisk-Holmberg *et al*., 1984). The remainder is slowly biotransformed by side chain de-ethylation in the liver leading to the formation of the major metabolite, desethyl-chloroquine which is the primary amine that can undergo deamination to form an alcohol - the 4-hydroxyl compound which then undergoes oxidation to form the carboxylic acid derivatives. The principal metabolite, desthyl-chloroquine also exhibits potent antimalarial activity against sensitive isolates of *P. falciparum* (Oduola *et al*., 1989), but less active than the parent drug and is also eliminated more slowly (Gustafsson *et al.*, 1987). The terminal elimination half-life is approximately 1-2months but, in terms of curative treatment (blood concentration), the real half-life ($t_{1/2}$) is about 6-7 days (Frisk-Homberg *et al*., 1984). Various half-lives have been reported after oral administration of chlroquine. This is because the determination of the half-life of a drug depends on the identification of the true terminal log linear elimination phase, which is difficult to obtain with chloroquine in view of its continuous redistribution from tissues to plasma over weeks. It also depends on the sampling time and sensitivity of the assay method. Drugs that inhibit the actions of liver microsomal enzymes prolong the half-life of chloroquine (Bowman & Rand, 1980).

(v) Toxicity: Serious adverse reaction associated with use of chloroquine is rare at therapeutic dosage. The common side effect among Nigerians is pruritus (Sowunmi *et al*, 1989). Some patients may vomit and may complain of blurred vision (Ferreras *et al*., 2007). It causes disruption of lysosome in living cells (Michihara *et al*., 2005). Cardiovascular abnormalities such as hypotension or cardiac arrhythmia progressing

to cardiac arrest and death are often observed after parenteral administration or overdose with the drug (Olatunde, 1970; Williams, 1966; Tuboku–Metzger, 1964; White, 2007). For instance, there are reports of sudden death following administration of intramuscular chloroquine to children with severe malaria (Olatunde, 1970). Since chloroquine is rapidly absorbed in the gastrointestinal tract, the use of parenteral route of administration is not encouraged. Prolonged treatment with chloroquine may cause a lichenoid skin eruption in some patients. The condition is mild and subsides when the drug is discontinued. Chloroquine overdose (usually self poisoning) is manifested by coma, convulsion, dysrhythmias and hypotension. Diazepam is a specific antidote (Riou *et al*., 1988). Oral activated charcoal is administered when an overdose is used.

Sulphadoxine

Sulfadoxine (SDX) is a weak acid (Fig. 2.9) and 88 - 90% bound to plasma protein, mainly albumin (Abdi *et al*., 1995; Mayxay *et al*., 2001). Body weight and age significantly influence the pharmacokinetics of sulphadoxine (Trengue *et al*., 2004). Sulfonamides are structural analogues and competitive antagonists of p-aminobenzoic acid (Rang *et al* 1995). Sulphadoxine inhibit dihydropteroate synthase (DHPS), a key enzyme in the folate biosynthesis (Rang *et al* 1995). Sulphadoxine is a partner drug with pyrimethamine for treating malaria. They act through a two steps synergistic blockage of *plasmodial* metabolism. The success of SP depends on this synergy: when either component is compromised, the effectiveness is dramatically reduced.

(i) Mode of action: This is the most used of a family of drug combinations which antagonize parasite folic acid synthesis. Sulphadoxine act by inhibition of dihydropteroate synthase (sulphonamide and sulphones) (Fig 2.10) This leads to a decrease level of fully reduced tetrahydrofolate, a necessary co-factor important in one-carbon transfer reactions in the purine, pyrimidine and amino acid biosynthetic pathway (Ferone, 1977). The lower level of tetrahydrofolate result in decreased conversion of glycine to serine, reduced methoinine synthesis, and lower thymidylate levels with a subsequent arrest of DNA replication (Gregson & Plowe, 2005).

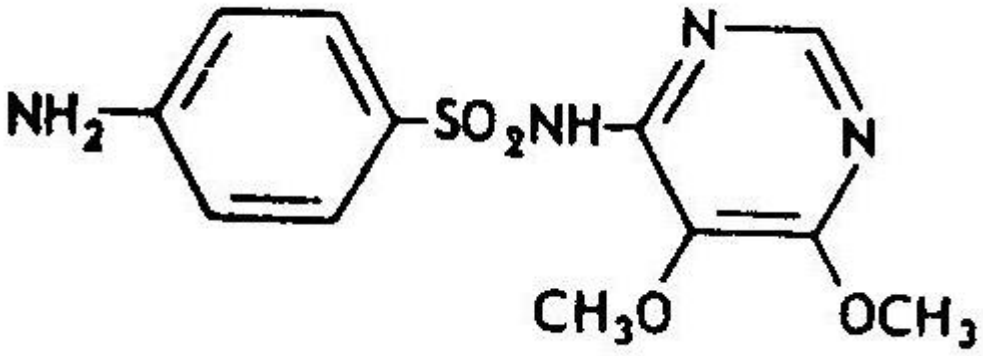


Figure 2.9: Chemical structures of Sulphadoxine.

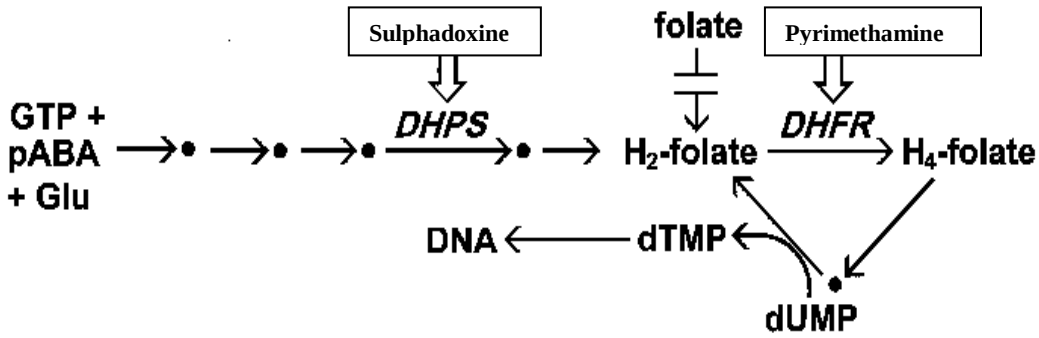


Figure 2.10: Mode of action of sulphadoxine/pyrimethamine

Pharmacokinetics of sulphadoxine

(i) *Absorption*: Sulphadoxine is well absorbed orally. In healthy subjects, peak plasma concentration is reached in 2-8h (Ahmad & Rogers, 1980; Weidekamm *et al.*, 1982; Dzinjalamala *et al.*, 2005a). Absorption in uncomplicated malaria is similar to that in healthy subjects (Winstanley *et al.*, 1992).

(ii) *Disposition:* The apparent volume of distribution of sulfadoxine (0.1-0.2L/kg) (WHO, 2000). It is lipophilic. Plasma protein binding of sulphadoxine in healthy subjects is high (88%) (Abdi *et al.*,1995). Red blood cells concentration of sulphadoxine is less than half of that in plasma (WHO, 2000). Pregnancy has been shown to have significant effect on disposition of sulphadoxine; it reduces SDX half-life, lowers the AUC and clearance is significantly greater than in non-pregnant women (Green *et al*., 2007).

(iii) *Elimination:* Sulphadoxine is a slowly eliminated sulfonamide. Unlike other sulphonamides, only 5% of sulphadoxine is n-acetylated and eliminated in the urine in this form (WHO, 1984). Biotransformation and clearance is approximately

0.5mL/kg/h. Elimination half-life of sulphadoxine ranges from 4.8 to 10.6 day (Winstanley *et al*., 1992; Dzinjalamala *et al.*, 2005).

(iv) *Toxicity*: Sulphadoxine is very well tolerated and severe adverse effects are uncommon (WHO, 1985). It has been reported to cause kernicterus in neonates but sulphonamide treatment in a lactating woman does not pose a threat to her breast fed neonates unless there is jaundice, prematurity or G-6PD deficiency (WHO, 2000). Life threatening erythema multiforme (Steven Johnson syndrome) and toxic epidermal necrolysis has been reported in individuals taking the drug for prophylaxis (Miller *et al.*, 1986).

Pyrimethamine

Pyrimethamine (PYR) is a base (Bergqvist *et al*., 1985)(Fiig. 2.11). Pyrimethamine is a competitive inhibitor of dihydrofolate synthesis (DHFR) (Peter, 1997). Pyrimethamine acts through a two steps synergistic blockage of *plasmodial* division. The success of pyrimethamine in treatment of malarial depends on sulphadoxine-pyrimethamine synergy: when either component is compromised, the effectiveness is dramatically reduced.

(i) Mode of action: This is the most used of a family of drug combinations which antagonize parasite folic acid synthesis. They act by sequential inhibition dihydrofolate reductase (pyrimethamine and biguanides) enzymes in the folate pathway (Fig 2.6) (Bzik et al., 1987; Triglia and Cowman, 1994; Triglia *et al*., 1999; Cowman, 1997). This leads to a decrease level of fully reduced tetrahydrofolate, a necessary co-factor important in one-carbon transfer reactions in the purine,

pyrimidine and amino acid biosynthetic pathway (Ferone, 1977). The lower level of tetrahydrofolate result in decreased conversion of glycine to serine, reduced methoinine synthesis, and lower thymidylate levels with a subsequent arrest of DNA replication (Gregson & Plowe, 2005).

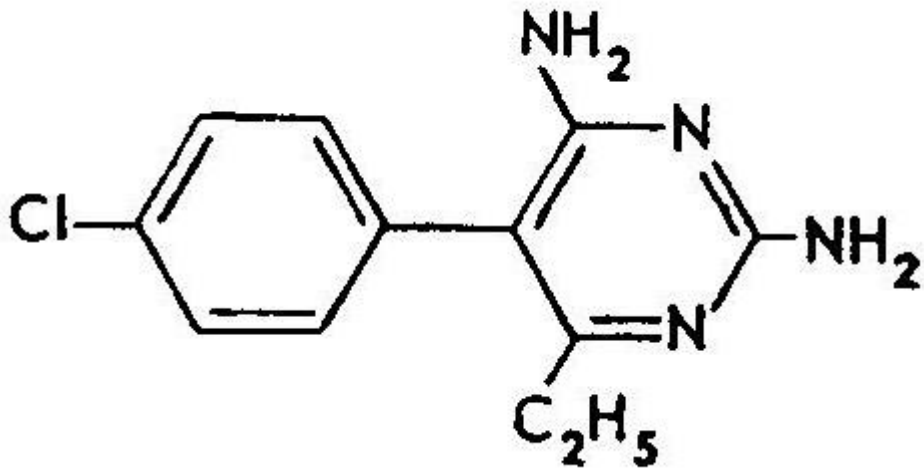


Figure 2.11: Chemical structures of Pyrimethamine

Pharmacokinetics of Pyrimethamine

(i) ***Absorption*****:** Pyrimethamine is well absorbed orally. In healthy subjects, peak plasma concentration is reached in 2-8h (Ahmad & Rogers, 1980; Weidekamm *et al*., 1982; Dzinjalamala *et al.,* 2005a). Absorption in uncomplicated malaria is similar to that in healthy subjects (Winstanley *et al.,* 1992).

(ii) ***Disposition:*** The apparent volume of distribution of pyrimethamine is 2-3 L/kg (Weidekamm *et al*., 1987). This is considerably larger than volume of the sulfadoxine (0.1-0.2L/kg) (WHO, 2000). Both drugs are lipophilics. Plasma protein binding of pyrimethamine in healthy subjects is high (93%) (Abdi *et al.*,1995).

(iii) ***Elimination:*** Biotransformation and clearance is approximately 0.5mL/kg/h. Elimination half-life of pyrimethamine is 3.3 to 4.8 day (Winstanley *et al*., 1992; Dzinjalamala *et al.,* 2005). Pyrimethamine is transformed to several unidentified metabolites and is cleared predominantly by hepatic biotransformation.

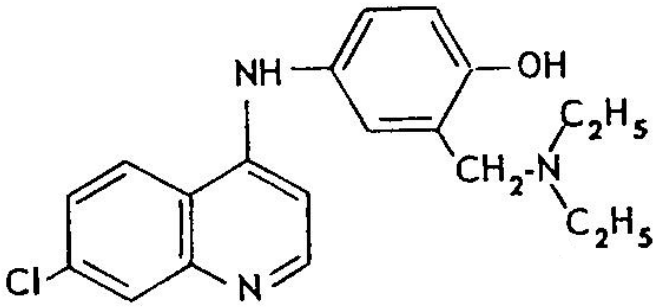
(iv) ***Toxicity*****:** Pyrimethamine is very well tolerated and adverse effects are rare (WHO, 1985). Prolong use of pyrimethamine may provoke folate deficiency in vulnerable subjects (pregnant or malnourished patients. No record of cardiotoxicity of pyrimethamine has been recorded either in animal or human experiment (White, 2007).

Amodiaquine

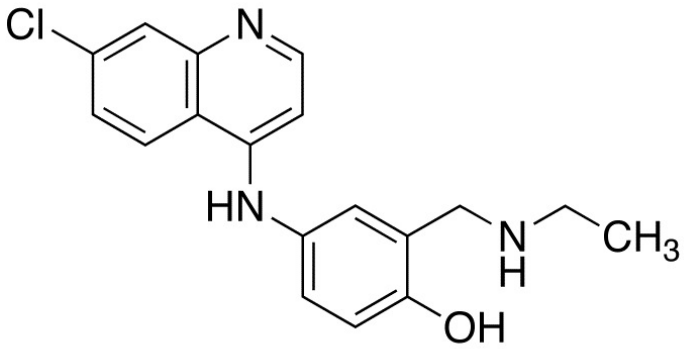
Amodiaquine is a 4-aminiquinoline similar to chloroquine (Fig 2.11). Amodiaquine was synthesized during the 2nd world war and has a similar action with chlroquine since both are structural analog (Brykier *et al*., 1988). It is made up of the same quinoline nucleus with lateral para-hydroxylphenyl group linked to a methyl-aminodiethyl chain. Amodiaquine is as effective as chloroquine and also used against chloroquine-resistant strains of *P. falciparum* (Van Dillen *et al*., 1999; Olliaro *et al*., 1996).

Mode of action of amodiaquine

Amodiaquine is a schizonticidal antimalarial drug. The mechanism of action of amodiaquine is not completely certain. Like other quinoline derivatives, it is thought to inhibit heme polymerase activity. This results in accumulation of free heme, which is toxic to the parasites and leads to the parasite death.



Amodiaquine



N-desethyl amodiaquine

Figure 2.12: Chemical structure of amodiaquine and N-desethyl amodiaquine

Pharmacokinetics of amodiaquine

(i) Absorption: Amodiaquine is rapidly absorbed, widely distributed and extensively metabolized to a pharmacologically active metabolite, desethyl amodiaquine, following oral administration and it seems that this metabolite is responsible for the antimalarial activity of the compound (Winstanley *et al*., 1985). The main metabolite of AQ is N-desethylamodiaquine (DEAQ) with other minor metabolites being 2-hydroxyl-DEAQ and N-bisdesethylAQ (bis-DEAQ) (Churchill *et al*., 1985, 1986; Mount *et al*., 1986). The primary route of AQ metabolism to DEAQ is via polymorphic CYP2C8 enzyme (Li *et al*., 2002; Adejei *et al*., 2008). The absorption

and elimination of amodiaquine in children appears to be similar to that in adults (WHO 2000).

(ii) ***Distribution:*** Amodiaquine is concentrated in the red blood cells, the whole blood to plasma concentration ratio being 3.1. The $AUC_{0\text{-}24h}$ for DEAQ in whole blood is significantly higher than that in plasma (Winstanley *et al*., 1987). The total apparent volume of distribution of DEAQ is small compared to chloroquine (87.9 – 243.1L/kg) (Stepniewska *et al*., 2009; Orrell *et al*., 2008). Both amodiaquine and DEAQ were found to be highly bound to plasma protein with a mean bound fraction of 92 and 85% respectively (Li *et al*., 2003).

(iii) Elimination: The formation of DEAQ is rapid, its elimination is very slow with a terminal half-life of over 100 h (Winstanley *et al*., 1987; Laurent *et al*., 1993; Adjei *et al*., 2008; Orrell *et al*., 2008). Excretion of DEAQ is slow and is detectable in urine 5 months after dosing (Wintsanley *et al*., 1987).

(iv) Toxicity: Amodiaquine at usual doses has similar adverse effect to chloroquine but high prevalence of agranulocytosis and hepatitis (Hatton *et al*., 1986) after long-term use. Hepatitis has been observed to occur from as early as 3 weeks (exposure to 3 weekly doses) to as late as 10 months of prophylaxis. Patients with severe amodiaquine induced hepatitis may remain jaundiced for 3-6 months. Severe neutropenia may occur if amodiaquine is used in anti-inflammatory doses for rheumatoid arthritis. It seems to be an unstable molecule and undergoes auto-oxidation in aqueous solution to yield a quinoneimine, which may be implicated in the drug's toxicity (Maggs *et al*., 1987). However, the adverse reactions appear to be idiosyncratic and have not been described when amodiaquine is used in malaria therapy (Olliaro *et al.*, 1996).

2.5: Antimalarial Drug Resistance

The emergence and spread of drug resistant malaria globally has become one of the most important problems in malaria control in recent years (Bradley, 1996; Olliaro *et al*., 2004). The parasites that caused the disease have developed resistance to all antimalarial including artemisinin derivatives (WHO 2006; Jambou *et al*., 2005; Dondorp *et al*., 2009). Chloroquine resistance is now common in every region where *P. falciparum* occurs (Edward & Biagini, 2006). Chloroquine was formerly replaced by sulphadoxine-pyrimethamine until when this combination succumbed in South-east Asia, South America and most recent, Africa (WHO 2006). The impact of resistant malaria is considerable and compounds the seriousness of malaria related morbidity and mortality (Price *et al*, 2001). It is the most critical factor in reducing the useful life span of a drug and undermining the drug policy.

Antimalarial drug resistance is defined as the ability of a parasite strain to survive and/ or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the tolerance of the subject; and the drug in question must gain access to the parasite or the infected red blood cell for the duration of time necessary for its normal action (WHO, 2001). The main mechanism underlying the development of resistance includes, naturally occurring genetic mutations in the malaria parasite. These mutations result in a decline in drug sensitivity depending on the class of antimalarial drug (Olliaro, 2004). Inadequate treatment (e.g. sub-therapeutics dose, sub-optimal drug) of a high biomass infection will not kill the mutant parasites and is the main selective pressure for resistance. Resistant parasites are then transmitted to other individuals by mosquitoes. In addition, drugs with long half-lives are more likely to select for resistance because low drug concentrations linger on and are only able to kill sensitive parasites (Nzila

et al., 2000).

The need to extend the clinical use of chloroquine has become important for several reasons including the socioeconomic situation in endemic areas of Africa and South America. The availability, tolerability and it low cost which had been the main cause to retain it in malaria control efforts. In Nigeria, chloroquine and sulphadoxine-pyrimethamine were antimalarial drugs of choice prior to change of treatment policy to artemisinin based combination therapy (ACTs) in 2004 (FMH, 2004). In few malaria endemic areas such as Malawi, the efficacy of CQ has been renewed through withdrawal of CQ from circulation to reduce drug pressure (Laufer *et al.*, 2006). Chloroquine has also been combined with artesunate yielding high efficacy (Fehintola and Balogun 2010). Sulphadoxine-pyrimethamine is currently employed as Intermittent Preventive Treatment in children (IPTc) and pregnant women (IPTp). It is essential to study the effect of drug resistance on pharmacokinetics of CQ or SP during acute infections in children. With respect to drug level estimation, the determination of antimalarial drug levels has been estimated in general from whole blood, plasma or red cell (White., 1992; Gbotosho *et al*., 2009; Obua *et al*., 2008).

However, antimalarials have also been estimated in saliva, for example, quinine (Salako and Sowunmi 1992; Wilson *et al.*, 1993, Babalola *et al* 1996). Although, there is much difficulty in measuring artemisinin drugs in biological samples, it is often easier to measure their partner drugs, e.g. amodiaquine or sulphadoxine plus pyrimethamine (Gitau *et al*., 2004; Gbotosho *et al*., 2009; Obua *et al*., 2008). An ideal medium from which antimalarial drugs should be measured should be non-invasive with respect to sample collection; saliva is one such medium (Salako and Sowunmi 1992; Wilson *et al*., 1993). This will provide useful

information that may guide drug combination strategies and drug therapeutic monitoring in order to revisit existing but abandoned drugs as a result of drug resistance to all antimalarial drugs (Dondorp *et al.*, 2009).

CHAPTER THREE

MATERIALS AND METHODS

Study 1: Studies on risk factors contributing to delay in parasite clearance in children with acute uncomplicated falciparum malaria.

Drug resistance in *Plasmodium falciparum* is common in many endemic countries but there is no clear recommendation on when to change therapy when there is delay in parasite clearance after initiation of therapy in African children. This study reports the relationship between delay in parasite clearance and anti-malarial treatment failure in children with falciparum malaria in an area of intense transmission in south-western Nigeria, where resistance in *Plasmodium falciparum* to CQ and SP has increase steadily over the past ten years.

3.0: Patients and drug treatment.

The studies were conducted between April 2002 and July 2010 in patients presenting at the Malaria Research Laboratories Clinic, University College Hospital, Ibadan. Ibadan is an hyper-endemic area for malaria in southwestern Nigeria (Salako *et al.*, 1990). Ethical clearance was provided by University of Ibadan/University College Hospital ethics committee and Oyo State Ministry of Health, Secretariat Ibadan. During the period, a series of antimalarial drug efficacy studies were conducted to evaluate the efficacy and safety of different treatment regimens (Table 3.1). The study was conducted with the assistance of a physician (Professor A. Sowunmi).

Briefly, children with symptoms compatible with acute uncomplicated falciparum malaria who fulfilled the following criteria were enlisted in the study: age ≤12 years, pure *P. falciparum* parasitaemia greater than 2000 asexual forms/µl blood, negative urine tests for antimalarial drugs (Dill-Glazko and lignin tests), absence of concomitant illness, no evidence of severe malaria (WHO, 2000) and written informed consent given by parents or guardians. Clinical and parasitological evaluation was done at enrollment (day 0) and on days 1-7, 14, 21 and day 28. In patients who received ACTs, follow-up was for 42 d. Clinical evaluation consisted of a general clinical examination including measurement of weight, core temperature and physical examination.

3.1: Sample collection.

Prior to treatment, 100µl of capillary blood sample was obtained from each patient and spotted on filter paper (110mm Whatman Filter paper) for determination of chloroquine or sulphadoxine levels. During follow-up, on day 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 thick blood films were prepared and 100µl of capillary blood samples were obtained and spotted on filter paper. The filter paper samples were stored in desiccated reseal able plastic bags at room temperature for measurement of chloroquine and sulphadoxine concentrations.

Table 3.1: Antimalarial drugs and their treatment regimens with time of study administered to children with acute uncomplicated falciparum malaria.

Treatment Group	Drugs*	Regimens†	No of patients	Years of study
Monotherapy	AQ	30 mg/kg of amodiaquine base over 3 days, that is, 10 mg/kg daily	573	2005/6
	AS	Artesunate given as 28 mg/kg over 7 days, that is, 4 mg/kg daily	120	2006
	CQ	30 mg/kg of chloroquine base over 3 days, that is, 10 mg/kg daily	388	2002-4
	MQ	Mefloquine given as 25mg/kg at presentation	176	2007/8
Artemisinin Combination Therapy	AQAS	Artesunate given as 4 mg/kg daily for 3 d plus amodiaquine given as in AQ above	142	2004/5/10
	AMQ	Mefloquine given as 25mg/kg at presentation plus artesunate as given in AQAS above	174	2006
	AL	Artemether (20mg) plus lumefantrine (120mg) given thus: 5-14kg received 1 tab., 15-24kg received 2 tab., 25-34kg received 3 tab., > 34kg received4 tab. at presentation, 8 h later and at 24, 36, 48 and 60 h after first dose	90	2007/8
Non-Artemisinin Combination Therap	AQSP	Amodiaquine given as in AQ above plus sulphadoxine-pyrimethamine given as 25 mg/kg of the sulphadoxine component at presentation	69	2002-4
	AQSFP	Amodiaquine given as in AQ above plus sulfalene-pyrimethamine given as 25 mg/kg of the sulfalene component at presentation	91	2006
	COT	Co-trimoxazole given as 20 mg of the sulphamethoxazole component twice daily	104	2003
	CQCP	30 mg/kg of chloroquine base over 3 days, that is, 10 mg/kg daily plus chlorpheniramine 8mg start and 4 mg 8 hourly for 5 d.	315	2003
	CQKET	30 mg/kg of CQ base over 3 days, i.e., 10 mg/kg daily plus ketotifen 25mg/kg start followed by 0.125 mg/kg 8 hourly for 4 d.	70	2004
	CQSP	30 mg/kg of chloroquine base over 3 days, that is, 10 mg/kg daily plus sulphadoxine-pyrimethamine given as 25 mg/kg of the sulphadoxine component at presentation	107	2007/8
	SP	Sulphadoxine-pyrimethamine given as 25 mg/kg of the sulphadoxine component at presentation	291	2003
	SPPB	Sulphadoxine-pyrimethamine given as in SP above plus probenecid at 20-25mg/kg in two divided doses daily for 3 day	42	2003

† All drugs were administered orally. AQ, amodiaquine; AQAS, amodiaquine plus artesunate; AQSP, amodiaquine plus sulphadoxine-pyrimethamine; AQSFP, amodiaquine-sulfalene-pyrimethamine; AMQ, mefloquine plus artesunate; AL, artemether plus lumefantrine; AS, artesunate; COT, co-trimoxazole; CQ, chloroquine; CQCP, chloroquine plus chlorpheniramine; CQKET, chloroquine plus ketotifen; CQSP, chloroquine plus sulphadoxine- pyrimethamine; MQ, mefloquine; SP, Sulphadoxine-pyrimethamine; SPPB, sulphadoxine-pyrimethamine plus probenecid;

3.2: Assessment of parasitaemia.

Thick and thin blood films prepared from a finger prick were Giemsa-stained and were examined by light microscopy under an oil-immersion objective, at X100 magnification and re-examined by an independent microscopist. Parasitaemia in thick films was estimated by counting asexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/μL of blood. Gametocytes were also counted in thick blood films against 1000 leukocytes assuming an average leukocyte count of 6000/μL of blood (Shaper & Lewis, 1971; Ezeilo, 1971; Sowunmi *et al.*, 1995).

3.3: Evaluation of response to drug treatment.

Response to drug treatment was assessed using World Health Organization (WHO) criteria (WHO, 1973 or 2003) as shown in Table 3. 2. In those with sensitive or RI response, parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitaemia for at least 72 h. Delay in parasite clearance was defined as a parasite clearance time > 2 d, and was based on the asexual life cycle of 48 h in the infected erythrocyte (White, 1997).

Table 3.2: Evaluation of clinical response of children to treatment with antimalarial drugs

Treatment Response	Description
S (sensitive)	clearance of parasitaemia without recurrence
RI (mild resistance)	parasitaemia disappears but reappears within 7 to 14 days;
RII (moderate resistance)	decrease of parasitaemia but no complete clearance from peripheral blood;
RIII (severe resistance)	no pronounced decrease or increase in parasitaemia at 48 hours after treatment
OR	
ACPR (adequate clinical and parasitological response)	if there was no reappearance of parasites or fever by day 14
ETF (Early treatment failure)	if danger signs or severe malaria developed on days 1, 2 and 3 in the presence of parasitaemia, or parasitaemia on day 2 higher than day 0 irrespective of temperature, or parasitaemia on day 3 with fever ≥37.5^{o}C, or day 3 parasitaemia ≥25% of the count on day 0
LCF (Late clinical failure)	if danger signs or severe malaria developed after day 3 in the presence of parasitaemia or presence of parasitaemia and fever (≥37.5^{o}C) on any day between day 4 and day 14;
LPF (Late parasitological failure)	if there was parasitaemia on day 14 and axillary temperature less than 37.5^{o}C without meeting any of the previous conditions

Study 2: Pharmacokinetic determinants of response to treatment with chloroquine in children with acute uncomplicated *Plasmodium falciparum* malaria.

Resistance to antimalarial has become an important issue in infectious diseases research. The usefulness of the common antimalarials, such as chloroquine and sulphadxoine-pyrimethamine is fading out as a result of resistance to the drugs. Apart from the issue that the parasites are resistant to these drugs, the way individuals respond to drug therapy varies considerably within a population. There are interindividual differences in absorption, distribution, metabolism and elimination of drug.

Monitoring of efficacy and evaluation of the pharmacokinetic parameters requires routine collection of whole blood for drug analysis. The use of whole blood collected through finger prick and adsorbed on filter paper has reduced risk of infection (Green *et al*., 2002) and semi-skilled field workers can collect the samples after minimal training with little discomfort or risk to the patient or volunteer. In addition, blood collection on filter paper reduces the need to provide facilities for separating and storing blood samples in the field. However, collection of field samples for pharmacokinetic studies in children has particular challenges since frequent sampling over a follow up period is required. Children tend to be less willing to have repeated finger pricks. When such situations arise, it may be difficult to obtain the correct blood volumes especially using capillary tubes. Different methods for measuring drug levels on filter paper have been developed (Minzi *et al*., 2003; Gitau *et al*., 2004; Hoegberg *et al*., 2005).

The pharmacokinetic determinants of antimalarial treatment failure are important in chemotherapy. This will help in prompt treatment of drug failure especially in children and pregnant women who are the vulnerable groups. It is

important to recommend correct optimal dosage regimen where it is insufficient especially in children and pregnant women in who low drug levels have been clearly documented (Barnes *et al*., 2008; Gbotosho *et al* 2009). Sub-therapeutic concentrations and variability in pharmacokinetic disposition to antimalarial drugs contribute to poorer responses to treatment and increase the spread of antimalarial drug resistance. Suboptimal drug concentrations which could result from inadequate drug absorption, an unusually large apparent volume of distribution or due to rapid clearance of the drug. Response to treatment is ensured if antimalarial drugs concentrations produce maximum effect until all malaria parasites are eliminated.

The use of Pharmacokinetic parameters to predict treatment outcome is important and this has not been utilised in antimalarial drug clinical trials to asses progression of responses to drug resistance. Routine measurement of drug concentrations of a slowly eliminated antimalarial drug such as CQ can be used to determine the minimum therapeutic concentration during treatment. For a drug eliminated slowly, the area under blood or plasma concentration time curve (AUC) could be a useful pharmacokinetic predictor of treatment outcome in uncomplicated malaria because it captures both the drug concentration and duration of exposure (White *et al*., 2008). The AUC comprises both the absorption and the elimination phases of a drug and provides a measure of parasite exposure to the anitmalarial drugs.

3.4: Study population

From the delay in parasite clearance study, it was shown that children who were treated with chloroquine and sulphadoxine-pyrimethamine had highest proportions of delay in parasite clearance 70.8% (CQ) and 63.9 % (SP). A cohort of children was randomly selected during enrolment for identification of

pharmacokinetic determinants of responses to chloroquine and assessment of pharmacokinetic disposition of sulphadoxine.

In this study, children, aged between 6 months – 12years were treated with standard doses of chloroquine (30 mg/kg of chloroquine base over 3 days, that is, 10 mg/kg daily). Children had microscopically confirmed infection with pure *P. falciparum* at parasiteamia greater than or equal to 2000 asexual parasites per micro liter of blood before enrollment. Prior to treatment, 100µl of capillary blood sample was obtained from each patient and spotted on filter paper (110mm Whatman filter paper) for determination of chloroquine or sulphadoxine level. During follow-up, on day 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 thick blood films were prepared and 100µl of capillary blood samples were obtained and spotted on filter paper. The filter paper samples were stored in desiccated resealable plastic bags at room temperature for measurement of chloroquine and sulphadoxine concentrations.

3.5: Drug analysis

3.5.1: Reagent for analysis.

Chloroquine, Desethyl chloroquine and Papaverine reference standard were obtained from Walter Reed Army Institute, USA; Diethyl ether, Sodium hydroxide, Hydrochloric acid and perchloric acid were obtained from BDH chemical England; Acetonitrile, Methanol and Water were HPLC grade obtained from LiChrosolv®, E. Merck, Germany. All other reagents were analytical grade.

3.5.2: Preparation of Stock and Working solutions.

1. Sodium hydroxide (NaOH, 5M) working solution was prepared by weighing 40g of NaOH pellets and dissolving in 200cm^3 of distilled water in a volumetric flask.

2. The working solution of Sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$, 0.02M) was made by weighing 3.1202g of the compound and dissolving in 1000cm^3 of distilled water.
3. Stock solution of chloroquine, desethyl chloroquine and papaverine (internal standard) were prepared by weighing 5mg of each of the drugs and dissolving in 5ml of 0.1M HCl. The working solution (100µg/ml) was prepared from 1mg/ml stock solution by making 1:10 dilution of 1mg/ml. One milliliter (1ml) of 1mg/ml stock was made up to 10ml with 0.1M HCl in a volumetric flask or centrifuge tube.
4. Preparation of 0.1M HCl was made from concentrated hydrochloric acid (HCl). The concentrated acid used had percentage purity of 35% with specific gravity of 1.18g/ml. The molarity of the concentrated HCl was calculated as follows;

% purity of acid =35% molar mass of HCl =36.5

Concentration of HCl in 1.18g/ml of the acid content = % purity x specific gravity = (35x1.18)/100g/ml = 0.413g/ml

i.e. 413 g of HCl in 1000ml (A)

Weight equivalent to molar mass of any compound in 1000ml of distilled water = 1M of the compound.

If 36.5g of HCl (was dissolved in 1000ml solvent) = 1M HCl (B)

Therefore if A=B

then, 413.0g (in 1000ml solvent) = (413g x 1M)/36.5g = 11.315M (C_1).

The molarity of the concentrated acid was 11.315M (C_1). To prepare 1000ml (V_2) of 0.1M HCl (C_2). The volume (V_1) of concentrated acid required can be

obtained from the formula, $C_1V_1 = C_2V_2$, where C_1 is initial concentration of the acid, C_2 is final concentration, V_1 volume of initial concentrated acid require and V_2 is final volume respectively.

$V_1 = (C_2V_2)/C_1$ = (0.1M X 1000ml)/11.315M

= (100ml)/11.315 = 8.837ml of concentrated acid.

Therefore, 0.1M was prepared by adding 8.837ml of concentrated acid to distilled water in volumetric flask and making up to 1000ml with distilled water.

3.5.3: Analysis of chloroquine from filter paper samples.

(a) Instrumentation.

Chromatographic separations were carried out on a Cecil Adept, High performance liquid chromatography unit. The unit consists of Cecil 4100 solvent delivery system with a Rheodyne valve injection system and a Cecil 4200 variable wavelength ultraviolet detector operating at 254nm with a Cecil 4900 chromatographic system manager. The separation was carried out on a Waters C_{18} Reversed-Phase 10µm µBondapak column of 3.9mm X 300mm dimension stainless steel maintained at room temperature.

(b) Mobile Phase Composition.

The mobile phase used for the analysis consisted of 0.02M phosphate buffer: methanol: acetonitrile (58:27:15 V/V/V) adjusted to pH 2.5 with perchloric acid. The mobile phase was delivered to the system at a flow rate 1ml/min.

(c) Extraction of chloroquine whole blood filter paper samples.

Whole blood concentrations of chloroquine were measured from filter paper blood spots using a modified HPLC analysis (Ogunbona *et al*, 1986). Briefly, filter paper sample was cut into pieces and the pieces were transferred into clean extraction tubes. The pieces of filter paper were soaked in 200µl of 0.1M HCl for 30 minutes. Two hundred nanogram (40µl of 5µg/ml) of papaverine as internal standard was added. Two hundred microlitres (200µl) of 5M NaOH was added to the content of each tube to basify the sample. A fixed volume (2ml) of diethyl ether was also added as the extraction solvent. The content was vortexed for sixty seconds and then centrifuged for 10 minutes at 2000g. After centrifugation the organic supernatant of the mixture was transferred into a clean centrifuge tube. One hundred microlitre (100µl) of 0.1M HCl was added to the organic supernatant and vortexed for 2 minutes. The mixture was thereafter centrifuged for ten minutes at 2000g. The organic supernatant was removed carefully and discarded. Twenty microlitre (20µl) of the aqueous phase was injected onto the column for analysis.

(d) Calibration Curves.

Calibration curve is a plot of detector response (peak/ratio) with change in concentration of known sample. It is a general method for determining the concentration of a substance in an unknown sample (blood) by comparing the unknown to a set of standard samples of known concentrations. Calibration curves based on peak-height ratios were prepared by spiking drug free blood sample with working solutions of chloroquine and desethyl-chloroquine to yield final concentration of 0 – 2000ng/ml for chloroquine and desethyl-chloroquine on filter paper. Chloroquine metabolite, desethyl-chloroquine was not considered because

desethyl-chloroquine standard powder was not available. The peak-height ratio for both the drug and the internal standard were recorded. Concentration of drug in the blood samples was extrapolated from the calibration curve as shown in Figure 4.4.

(e) Precision and percentage recovery of the analytical method.

Filter paper samples (drug free) were spiked with known concentration of chloroquine (between 0 - 2000ng/ml of chloroquine). A 40µl (of 5µg/ml) aliquot of internal standard was added to the cut filter paper in extraction tube. The samples were prepared (n=5), taken through the extraction procedure as described previously and injected onto the column. Another set of standard chloroquine solutions were prepared to yield actual concentration in the spiked filter papers. An aliquot (20µl) of the solution was injected directly into the column. The peak height of the extracted standard samples and the solutions injected onto the system were compared to obtain the percentage recovery of the method, intra-assay (within day) and inter-assay (day-to-day) coefficient of variation (C.V.). Limit of detection of the drug was also determined.

Study 3: Development of High Performance Liquid Chromatography analytical method for measurement of sulphadoxine concentrations in filter paper sample.

Sulphadoxine-pyrimethamine (SP) analysis from filter paper has been a very difficult analysis because of the nature of chemical composition for sulphadoxine and pyrimethamine. Sulphadoxine is both an acid and a weak base while pyrimethamine is a weak base (Green *et al*., 2002). This method was developed in order to reduce cost of analysis and the burden of sample storage especially during filed trials. Although this method analysed both sulphadoxine-pyrimethamine (SP) but only SDX was considered. It has been reported that there is a correlation between plasma concentration of SDX and PYR (Bergvist *et al*., 1987), however knowledge of sulphadoxine concentrations provides some information of the magnitude of the accompanied pyrimethamine concentration as well. The ratio of combination of sulphadoxine-pyrimethamine is 20:1 (500mg of sulphadoxine:25mg of pyrimethamine) in a tablet.

3.6: Analysis of sulphadoxine from filter paper samples.

a. Instrumentation.

The same HPLC unit as in CQ analysis was used for sulphadoxine analysis but the column used was different. The eluent was monitored using a UV detector operated at 240nm with a Cecil 4900 chromatographic system manager. The separation was carried out on a Beckman Coulter ODS 5µm column of 4.6mm X 15cm dimension stainless steel maintained at room temperature.

b. Preparation of Stock and Working solutions.

Stock solution of sulphadoxine, pyrimethamine and sulisoxazole (internal standard) were prepared by weighing 5mg of each of the drugs and dissolving in 5ml of 70% ethanol prepared with 0.1M HCl solution. Working solution (100µg/ml) was prepared from 1mg/ml stock solution by making 1:10 dilution of 1mg/ml. One milliliter (1ml) of 1mg/ml stock was made up to 10ml with 0.1MHCl in a volumetric flask or centrifuge tube.

c. Mobile phase composition.

The mobile phase used for the analysis consisted of 1% triethylamine solution containing 0.05M phosphate buffer: methanol: acetonitrile (70:17:13 V/V/V) adjusted to pH 3.4 with phosphoric acid. The mobile phase was delivered to the system at a flow rate of 0.9ml/min.

d. Measurement of sulphadoxine from whole blood on filter paper samples.

Sulphadoxine (SDX) extraction was performed by cutting filter paper samples into pieces and transferring the pieces into clean extraction tubes. The pieces of filter paper were soaked in 200µl of 0.1MHCl for 30 minutes and twenty microlitre (20µl) of 500µg/ml sulisoxazole as internal standard was added. Two millitres (2ml) of acetonitrile was also added as an extracting solvent. The content was vortexed for two minutes and then centrifuged for 10 minutes at 2000g. After centrifugation the acetonitrile supernatant of the mixture was transferred into a clean centrifuge tube and dried under a stream of nitrogen gas in a water bath at 50^{O}C. One hundred microlitres (100µl) of 0.1M HCl was added to reconstitute the dried extracted sample, vortexed for 2 minutes and centrifuged for ten minutes at 2000g. Twenty microlitres

(20µl) of the aqueous phases was injected onto the column for analysis. The retention time for sulphadoxine and sulisoxazole were determined.

e. Calibration curve for sulphadoxine.

The calibration curve for sulphadoxine was prepared by spiking drug free blood sample with working solutions of SDX to yield final concentration of 0 – 60µg/ml for sulphadoxine. Using an adjustable pipette, 100µl of drug-spiked donor whole blood was adsorbed onto the filter paper. The samples were placed in an incubator at 37^{o}C and allowed to dry overnight. The spiked filter paper blood sample were taken through the normal assay procedure as discussed above, and calibration graphs were constructed using the peak-height ratio of SDX to internal standard against concentration.

f. Precision and Recovery.

To assess precision, coefficient of variation (CV) was determined for intra- and inter assay variability. This was achieved by carrying out repeated analysis of drug-free whole blood spiked with different concentrations of sulphadoxine (n>5 for each concentration) on each of six days. Calibration curves were prepared from the measurement of peak height ratios of the SDX and internal standard. Extraction recovery was determined for each concentration by comparing peak heights ratio of the extracted known standards with the directly injected standard concentrations. Commonly used antimalarial drugs including chloroquine, mefloquine, quinine, sulphamethoxazole and trimethoprim were studied for interference by spiking the drugs in the blank whole blood. The drugs were extracted according to the method

described above. The presence of peaks was monitored after injection of 20µl of the reconstituted sample.

Study 4: Pharmacokinetic disposition of sulphadoxine in children with acute uncomplicated malaria treated with standard dose of sulphadoxine-pyrimethamine.

Sulphadoxine-pyrimethamine is currently recommended by WHO as a partner drug with artesunate in the chemotherapy of malaria and it is also being used alone for intermittent preventive treatment for malaria in pregnancy and in infants (WHO 2006; WHO 2007; May *et al.*, 2008). However, information on pharmacokinetics of SDX-pyrimethamine in children with acute uncomplicated malaria is limited (Barnes *et al.*, 2006; Dzinjalamala *et al* 2005; Obua *et al* 2008; Hellgren *et al* 1990; Winstanley *et al.*, 1992 and Aubouy *et al* 2003). Efforts in this study were thus devoted to studying the pharmacokinetic disposition of SDX and evaluating the effect of pharmacokinetic variability on therapeutic efficacy in children between the ages of 6 months and 12 years with acute uncomplicated falciparum malaria treated with standard dose of SP.

Seventy-four patients treated with standard dose of sulphadoxine-pyrimethamine (25 mg/kg of the sulphadoxine component) were randomly selected from cohort of children who had highest proportion of delay in parasite clearance. The simple cost-effective high performance chromatographic analytical method that was developed for the analysis of sulphadoxine in capillary whole blood collected on filter paper in study 3 was used for the analysis of these samples. Samples were collected before treatment (day 0), 1, 2, 3, 4, 5, 6, 7, 14, 21 and day 28 post treatment respectively. Reagent and chromatographic conditions are as stated in study 2 and study 3.

Study 5: Evaluation of the use of saliva for therapeutic drug monitoring in children with uncomplicated *Plasmodium falciparum* malaria treated with amodiaquine-artesuante.

Saliva offers an easily accessible and sometimes useful body fluid for therapeutic drug monitoring and for pharmacokinetic and pharmacodynamic studies. Saliva is limited to the unbound fraction of the drug. Thus, saliva levels may be more reflective of drug concentrations at the site of action than are total drug concentrations in plasma or whole blood. It has been shown that the saliva concentrations of particular drug can be used to predict how much of those drugs is in the blood (Fatah and Cohn, 2003).

3.7: Patients enrolled in the study and sample collection.

Patients were randomly selected from cohort of children who received oral doses of artesunate-amodiaquine (4mg/kg artesunate plus 30mg/kg amodiaquine base over 3 days). Seven children were selected from this group of children. Venous blood (5ml) was collected at 0hr before treatment, 4hr post treatment and thereafter on day 7, 14, 21, 28 and 35. Blood samples were collected into heparinised tube and were immediately centrifuged at 2000g for 10 minutes to separate the plasma and red blood cells, which were stored at -20^{O}C until analysed. Saliva samples were taken at the same times that venepuntures were done. The samples were collected after rinsing the mouth with water and without any special stimulus to increase saliva flow. The saliva (3-5ml) was immediately centrifuged and the clear fluid was removed and stored at -20^{O}C until analysed.

3.8: Analysis of amodiaquine or desethyl amodiaquine from plasma or saliva samples.

(a) Instrumentation.

Chromatographic separations were carried out on the same HPLC unit as in CQ study (study 2).

(b). Preparation of Stock and Working solutions.

Stock solution of amodiaquine, desethyl amodiaquine and quinidine (internal standard) were prepared by weighing 5mg of each of the drugs and dissolving in 5ml of 70% ethanol prepared with distilled water. Working solution (100µg/ml) was prepared from 1mg/ml stock solution by making 1:10 dilution of 1mg/ml. One milliliter (1ml) of 1mg/ml stock was made up to 10ml with distilled water in a volumetric flask or centrifuge tube.

(c) Mobile Phase Composition.

The mobile phase comprised 0.02M potassium diphosphate buffer, methanol and acetonitrile in the ratio 75:23:2 (V/V/V) adjusted to pH 2.65 with phosphoric acid. The separation was carried out on a Hypersil Reversed-Phase 5µ column of 4.6 mm x 250 mm dimension stainless steel maintained at room temperature. The UV detector was set at 254nm. The mobile phase was delivered to the system at a flow rate 1ml/min.

(d) Extraction of amodiaquine/desethyl amodiaquine from plasma or saliva sample.

Amodiaquine and desethylamodiaquine in plasma and saliva was assayed by a specific high performance liquid chromatography (HPLC) with ultraviolet detector

using a modification of a method described by Gitua and others (2004). Briefly, 0.5 ml of plasma or saliva was transferred into clean extraction tubes. Two hundred and fifty nanogramm (5µl of 50µg/ml) of quinidine was added as internal standard (IS). A fixed volume (5 ml) of diethylether was also added as the extraction solvent. The content was vortexed for sixty seconds and then centrifuged for 10 minutes at 2000g. After centrifugation the organic supernatant of the mixture was transferred into a clean centrifuge tube and dried in a fume cupboard at 40^{O}C. The residue was reconstituted in sixty-five microlitre (65µl) of mobile phase and fifty microlitre (50µl) injected onto the column.

(e) Measurement of saliva pH.

The pH of saliva was measured immediately after collection using a pH meter (Mettler-Toledo GmbH, Sonnenbergstrasse, Switzerland) and recorded immediately.

(f) Calibration Curves.

Stock solutions of amodiaquine, desethyl amodiaquine (1mg/ml) and internal standard quinidine (1mg/ml), was prepared in 70% methanol. Drug free plasma and saliva was spiked with the working solution containing both AQ and DEAQ to yield final concentrations ranging from 600 – 100ng/ml of AQ and DEAQ. The samples were assayed as described above and this was used for calibration curve. Concentration of drug in the plasma or saliva samples was extrapolated from a calibration curve.

(g) Precision and percentage recovery of the analytical method.

Drug free plasma or saliva samples were spiked with known concentration of AQ or

DEAQ (between 100 -800ng/ml) and 5µl of 50µg/ml aliquot of internal standard was added. The samples (n=5), were taken through the extraction procedures as described previously and injected onto the column. Another set of standard AQ and DEAQ solutions were prepared to yield actual concentration in the spiked plasma or saliva sample. An aliquot (20µl) of the solution was injected directly onto the column. The peak height of the extracted standard samples and the solutions injected onto the system were compared to obtain the percentage recovery of the method, intra-assay (within day) and inter-assay (day-to-day) coefficient of variation (C.V.).

3.9: Statistical analysis for all studies.

Data were analysed using version 6 of the Epi-Info software (Anon., 1994), and the statistical program SPSS for Windows version 10.01 (Anon., 1999). Proportions were compared by calculating χ^2 with Yates' correction. Normally distributed, continuous data were compared by Student's t-tests and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann-Whitney U-test and the Kruskal-Wallis test (or by Wilcoxon rank sum test). The values presented are generally means and standard deviations (s.d.) or standard error of mean (s.e.m.).

In order to determine the risk factors for delay in parasite clearance and pharmacokinetic factors associated with CQ treatment failure, the relationship between each of the clinical or parasitological data at enrolment and pharmacokinetic parameters following treatment was investigated by univariate analysis. A multiple logistic regression model was used to test the association between response to drug treatment (sensitive or resistant) and the factors that were significant in the univariate

analysis: age, presence of fever (temperature ≥ 37.5^{O}C) at presentation, PC > 2 d (yes or no at presentation or during follow up): presence of fever, asexual parasitaemia at presentation or during follow-up, a history of vomiting, and drug treatment. All these factors were used in multiple logistic regression analysis models to test the association with delay in parasite clearance.

GraphPad Prism (version 3.02) and Microsoft Excel statistical software's were used for collation of all the drug concentrations obtained from each patient and used for test of linearity of the standard curves. Pharmacokinetic parameters were obtained using Turbo Ken software (designed by the Department of Clinical Pharmacology, University of Southampton, United Kingdom). *P* values < 0.05 were considered significant for all statistical analysis except in multivariate analysis of $t_{1/2}$ where *P* < 0.3 was 'falsed' for the analysis. In comparison of disposition of amodiaquine and desethylamodiaquine in plasma and saliva in children with uncomplicated *Plasmodium falciparum* malaria, plasma and saliva concentration-time curve was plotted for each subject. Oral clearance was calculated from the equation CL= D/AUC, D is the dose given. The apparent volume of distribution (Vd) was calculated from the equation Vd=CL/β.

CHAPTER FOUR

RESULTS

Study 1: Risk factors contributing to delay in parasite clearance in uncomplicated falciparum malaria in children.

4.0: Study Population

Between April 2002 and July 2010, 2,752 children (1,342 females and 1,410 males) were enrolled into the antimalarial drug studies. All children who were recruited into these studies had primary infections with *P. falciparum*. There were 1,716 under five-year olds. The geometric mean parasitaemia at enrolment was 34,044 asexual parasites/µl of blood (95% CI 30,400 – 33,664) as shown in Table 4.1.

4.1: Drug treatment and delay in parasite clearance

Overall, delay in parasite clearance occurred in 1,237 of the 2,752 children (45%) (Figure 4.1 and Table 4.2). The highest proportions of children showing delay in parasite clearance were found in those treated with chloroquine (CQ), sulphadoxine-pyrimethamine (SP), chloroquine plus chlorpheniramine (CQCP), co-trimoxazole (COT), amodiaquine (AQ), chloroquine plus ketotifen (CQKET), chloroquine plus sulphadoxine-pyrimethamine (CQSP), amodiaquine plus sulphadoxine-pyrimethamine (AQSP), sulphadoxine-pyrimethamine plus probenecid (SPPB) and mefloquine (MQ). The proportions of children with delay in parasite clearance were significantly lower in those treated with AS, AQAS, AL and AMQ when compared with the latter group above (χ^2 = 447.91 df = 8, P < 0.0000001). There was no significant

difference in the proportions of children with delay in clearance in those treated with AS (3 of 120), AQAS (14 of 142), AL (6 of 90) and AMQ (10 of 174) (χ^2 = 6.1, df =3, P = 0.10). Infection in 291 of the 2,752 (10.6%) children failed to respond to treatment (Table 4.3).

Table 4.1: Baseline clinical and parasitological parameters of 2752 children with acute uncomplicated falciparum malaria enrolled in the study.

Variables	**Mean ± SD** (range)	95% CI
Age (year)	6.1 ± 3.0 (0.5-12)	6.0-6.2
No. < 5 years	1,716 (63%)	
Weight (kg)	17.3 ± 6.4 (5-47)	17.0-17.6
Axillary temperature (^{O}C)		
(n = 2428)	38.3 ± 1.2 (34-42)	38.2-38.3
No. with > 40^{o}C	210	
Haematocrit (%) (n = 994)	30.5 ± 4.8 (10-51)	30.1-30.7
No. with < 30%	380	
Parasitaemia (/µl) GM	34,044	30,400-33,664
Range	2009-1,194,285	
No with > 100,000 (/µl)	638 (23.2%)	
No. with > 250,000 (/µl)	187 (6.8%)	
Gametocytaemia (/µl) GM	27 (6 - 4188)	
Duration of illness (d)	3.0 ± 1.4 (1-14)	2.9-3.0
Duration of vomiting (d)	1.3 ± 1.4 (1-9)	1.2-1.3

GM, geometric mean; parasitaemia= asexual parasites/µl of blood

Table 4.2: Proportions of children with Delay in Parasite Clearance following treatment with standard doses of selected antimalarial drugs.

Drug	Proportion with delay in PC (%)
Total with delay in PC	45 (1237)
Chloroquine (CQ)	70.8
Sulphadoxine-pyrimethamine (SP)	63.9
Chloroquine + chlorpheniramine (CQCP)	60.0
Co-trimoxazole (COT)	58.6
Amodiaquine (AQ)	52.0
Chloroquine + Ketotifen (CQKET)	51.4
Chloroquine + Sulphadoxine-pyrimethamine(CQSP)	42.0
Amodiaquine + sulfalene-pyrimethamine (AQSFP)	37.6
Sulphadoxine-pyrimethamine + probenecid (SPPB)	30.9
Mefloquine (MQ)	22.7
Artesunate + amodiaquine (AQAS)	9.9
Artesunate + lumefantrine (AL)	6.6
Artesunate + Mefloquine (AMQ)	5.7
Artesunate (AS) (monotherapy)	2.5

† All drugs were administered orally. AQ, amodiaquine; AQAS, amodiaquine plus artesunate; AQSP, amodiaquine plus sulphadoxine-pyrimethamine; AQSFP, amodiaquine-sulfalene-pyrimethamine; AMQ, mefloquine plus artesunate; AL, artemether plus lumefantrine; AS, artesunate; COT, co-trimoxazole; CQ, chloroquine; CQCP, chloroquine plus chlorpheniramine; CQKET, chloroquine plus ketotifen; CQSP, chloroquine plus sulphadoxine- pyrimethamine; MQ, mefloquine; SP, Sulphadoxine-pyrimethamine; SPPB, sulphadoxine-pyrimethamine plus probenecid.

Table 4.3: Proportion of children with falciparum malaria who failed to respond to treatment with standard doses of antimalarial drugs.

Day	Total failure	Failure rate (%)
7	57	2.1
14	136	5.3
21	291	10.6

χ^2 for trend = 158, $p < 0.000001$; Infection in 291 of the 2,752 (10.6%) children failed to respond to treatment.

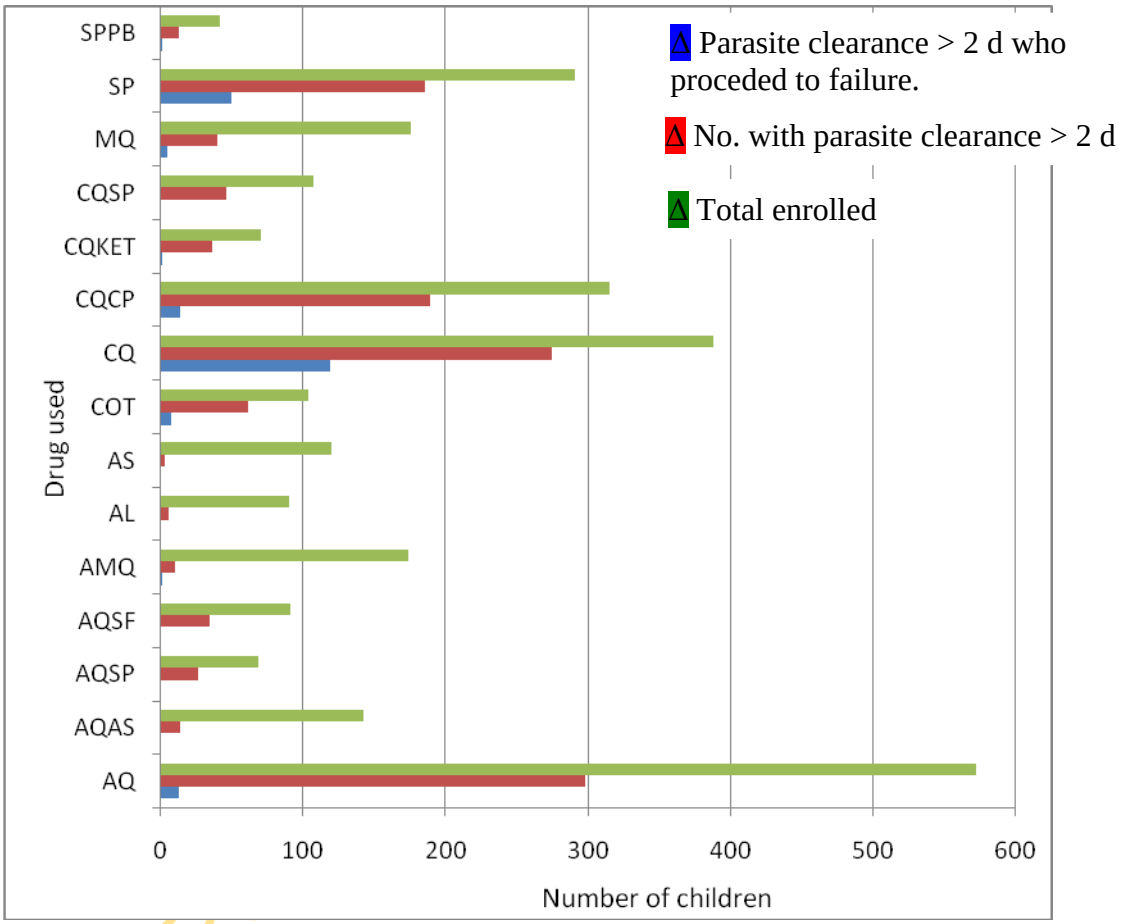


Figure 4.1: Numbers of children with delay in parasite clearance (PC > 2 d) following treatment with antimalarial drugs.

4.2: Risk factors for delay in parasite clearance at enrolment

The following were found to be independent risk factors for delay in parasite clearance at enrolment (Table 4.4): age ≤ 2 years (Adjusted odds ratio [AOR] = 2.13, 95% confidence interval [CI]1.44-3.15, P < 0.0001), presence of fever (AOR = 1.33, 95% CI = 1.04 –1.69, P = 0.019) and parasitaemia >50,000 asexual parasite/µl of blood (AOR = 2.21, 95% CI = 1.70 - 2.75, P < 0.0001). A history of vomiting was associated with an increased risk of delay in clearance (crude odds ratio = 1.34, 95% CI = 1.07 -1.58, P = 0.009).

4.3: Risk factors for delay in parasite clearance following initiation of treatment

Following treatment, a body temperature ≥ 38^{o}C and parasitaemia > 20,000 asexual parasite/µl of blood a day after treatment began, were independent risk factors for delay in clearance (Table 4.5). Non artemisinin monotherapy was associated with delay in clearance.

4.4: Delay in parasite clearance and treatment failure

Figure 4.2 shows the proportions of children treated with various antimalarials who had delay in parasite clearance and who subsequently failed treatment. Overall, 291 children failed treatment. Of these, 211 (72%) had delay in parasite clearance. The latter represent 17% of the total children (1237) with delay in parasite clearance. In summary 13 of 20, 7 of 10, 119 of 146, 14 of 15, 5 of 19 and 50 of 60 children treated with AQ, COT, CQ, CQCP, MQ, and SP respectively were those who failed treatment and previously had delay in parasite clearance.

Table 4.4: Predictors of delay in parasite clearance at presentation in children with acute falciparum malaria treated with antimalarial drugs.

Variables	Number enrolled	PC >2 d	Crude OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
Age (years)						
>2	2446	1076	1		1	
≤2	267	140	1.40 (1.09 –1.80)	0.008	2.13(1.44 –3.15)	<0.0001
Gender						
Female	1342	607	1			
Male	1240	557	0.98 (0.84 –1.15)	0.870	-	-
Fever*						
Absent	686	252	1		1	
Present	1743	842	1.6 (1.34 -1.93)	<0.0001	1.33 (1.04-1.69)	0.019
Duration of illness (d)						
≤ 3	2085	926	1			
> 3	521	246	1.12 (0.90 – 1.30)	0.225	-	-
Haematocrit (%)						
≥ 30	614	182	1			
<30	380	114	1.01 (0.76 – 1.34)	0.904	-	-
Parasitemia (/µl blood)						
≤ 50,000	1607	634	1		1	
> 50,000	1145	603	1.70 (1.46 –1.99)	<0.0001	2.21 (1.77 – 2.75)	< 0.0001
Gametocytemia						
Absent	2086	896	1			
Present	232	90	0.84 (0.63 – 1.10)	0.224	-	-
Vomiting						
No	1005	519	1		1	
Yes	665	387	1.34 (1.07-1.58)	0.009	1.21 (0.90-1.51)	0.089
Hepatomegaly						
Absent	471	237	1			
Present	798	430	1.54 (0.91 – 2.44)	0.219	-	-

CI, confidence interval; OR, odd ratio; PC, parasite clearance, *Body temperature ≥ 37.5°C

Table 4.5: Predictors of delay in parasite clearance on day 1 after treatment in children with acute falciparum malaria.

Variables	Number enrolled	PC >2 d	Crude OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
Axillary temperature (^{O}C)						
<38.0	2326	1012	1		1	
≥38.0	228	139	2.02 (1.53 -2.67)	<0.0001	1.80 (1.30-2.50)	< 0.001
Parasitaemia (/µl blood)						
≤ 20,000	1328	463	1		1	
>20,000	683	467	5.25 (4.20 – 6.48)	<0.0001	5.13 (4.14 – 6.35)	< 0.001
Drug treatment *						
CQ	388	275	1	1	1	
AQ	573	298	0.44 (0.33 – 0.58)	<0.0001	0.79 (0.63 – 0.98)	< 0.031
AQAS	142	14	0.05 (0.03 – 0.08)	<0.0001	0.24 (0.10 – 0.57)	< 0.0001
AQSP	69	26	0.24 (0.14 – 0.42)	<0.0001	0.44 (0.27 – 0.73)	0.002
AQSF	91	34	0.25 (0.15 – 0.39)	<0.0001	0.43 (0.29 – 0.68)	< 0.0001
AMQ	174	10	0.03 (0.01 – 0.05)	<0.0001	0.04 (0.02 – 0.09)	< 0.0001
AL	90	6	0.03 (0.01 – 0.07)	<0.0001	0.05 (0.02 – 0.12)	< 0.0001
AS	120	3	0.01 (0.00 – 0.03)	<0.0001	0.02 (0.00 – 0.06)	< 0.0001
COT	104	61	0.58 (0.37 – 0.91)	0.017	1.03 (0.68 – 1.36)	0.877
CQCP	315	189	0.61 (0.45 - 0.84)	0.002	1.09 (0.83 – 1.42)	0.512
CQKET	70	36	0.43 (0.25 – 0.73)	0.001	0.77 (0.47 – 1.25)	0.298
CQSP	107	46	0.31 (0.19 – 0.48)	<0.0001	0.54 (0.36 – 0.82)	0.004
MQ	176	40	0.12 (0.08 – 0.18)	<0.0001	0.21 (0.15 - 0.31)	< 0.0001
SP	291	186	0.72 (0.52 – 1.00)	0.055	-	-
SPPB	42	13	0.18 (0.09 - 0.37)	<0.0001	0.33 (0.17 -0.98)	0.001

CI, confidence interval; OR, odd ratio; PC, parasite clearance, * Values of OR represent chances of delay in parasite clearance. † All drugs were administered orally. AQ, amodiaquine; AQAS, amodiaquine plus artesunate; AQSP, amodiaquine plus sulphadoxine-pyrimethamine; AQSFP, amodiaquine-sulfalene-pyrimethamine; AMQ, mefloquine plus artesunate; AL, artemether plus lumefantrine; AS, artesunate; COT, co-trimoxazole; CQ, chloroquine; CQCP, chloroquine plus chlorpheniramine; CQKET, chloroquine plus ketotifen; CQSP, chloroquine plus sulphadoxine- pyrimethamine; MQ, mefloquine; SP, Sulphadoxine-pyrimethamine; SPPB, sulphadoxine-pyrimethamine plus probenecid;

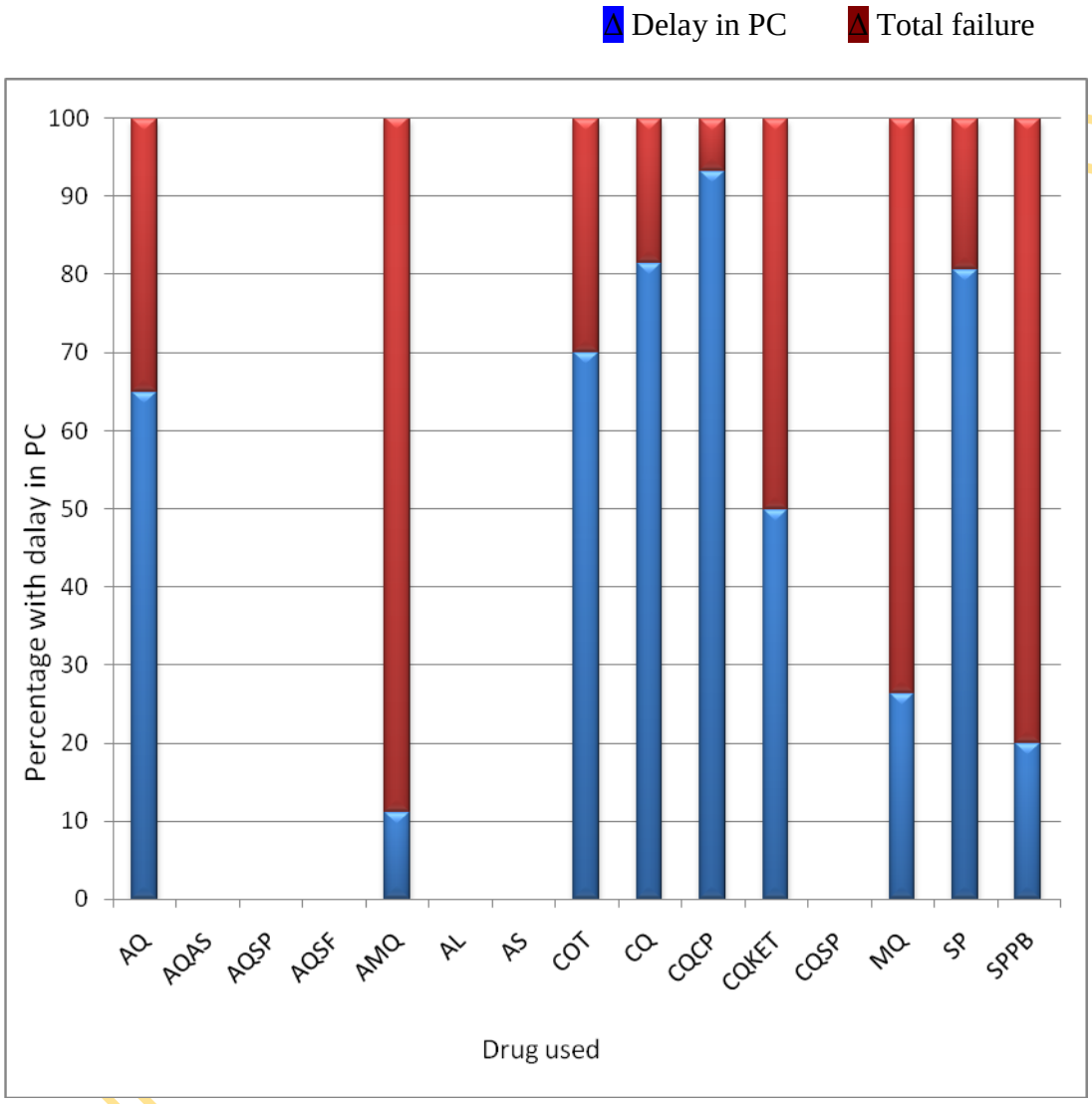


Figure 4.2: The proportions of children treated with various antimalarials who had delay in parasite clearance (PC) and subsequently failed treatment. (AQ, amodiaquine; AQAS, amodiaquine plus artesunate; AQSP, amodiaquine plus sulphadoxine-pyrimethamine; AQSFP, amodiaquine-sulfalene-pyrimethamine; AMQ, mefloquine plus artesunate; AL, artemether plus lumefantrine; AS, artesunate; COT, co-trimoxazole; CQ, chloroquine; CQCP, chloroquine plus chlorpheniramine; CQKET, chloroquine plus ketotifen; CQSP, chloroquine plus sulphadoxine-pyrimethamine; MQ, mefloquine; SP, Sulphadoxine-pyrimethamine; SPPB, sulphadoxine-pyrimethamine plus probenecid)

Study 2: Pharmacokinetic determinants of response to treatment with chloroquine in children with acute uncomplicated *Plasmodium falciparum* malaria.

Chloroquine was well resolved in the analysis using this method. There was a good linearity (r^2 = 0.9992; Figure 4.3 and Appendix Table 4.a) in the standard curve obtained for the analysis of chloroquine (CQ). In addition, the percentage recovery of CQ as well as the precision and accuracy of the analytical method for CQ are shown in Table 4.6 and 4.7. Figure 4.4 shows the chromatograms for CQ, desethyl chloroquine (DCQ) or papaverine (PPV), internal standard obtained during analysis. There was no interference with other drugs (e.g. paracetamol, amodiaquine and other commonly used drugs) with the peaks obtained (Figure 4.4). The limit of detection for chloroquine was 5ng/ml. The retention time for DCQ, CQ and the PPV were 5.5 minutes, 6.5 minutes and 10.5 minutes respectively.

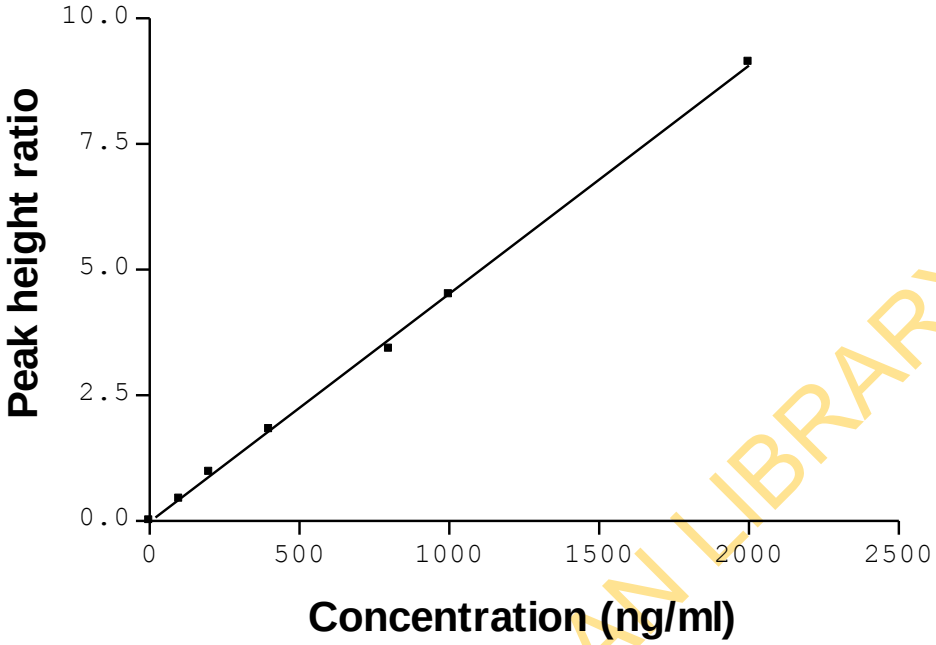


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Figure 4.3: Standard curve of chloroquine for extrapolation of unknown concentrations from whole blood spotted on filter paper. ($r^2 = 0.9992$)

Table 4.6: Percentage recovery of chloroquine from whole blood spotted on filter paper using the analytical method for chloroquine.

	Concentration (ng/mL)	% Recovery (±S.D)	n
Whole blood	200	85.6±5.9	5
	400	87.5±2.5	4
	800	89.6±1.9	4

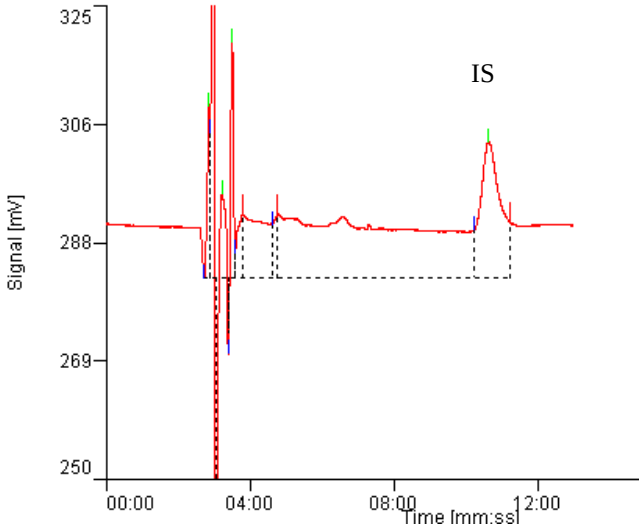
S.D= Standard deviation

Table 4.7: Precision of analytical method for extraction of chloroquine from whole blood spotted on filter paper.

	Concentration (ng/mL)	C.V (%)	n
Intra-assay	Chloroquine		
	100	2.9	5
	200	1.9	5
	1000	2.5	4
Inter-assay	Chloroquine		
	100	3.2	5
	200	3.1	6
	1000	2.8	4

C.V= coefficient variation

A



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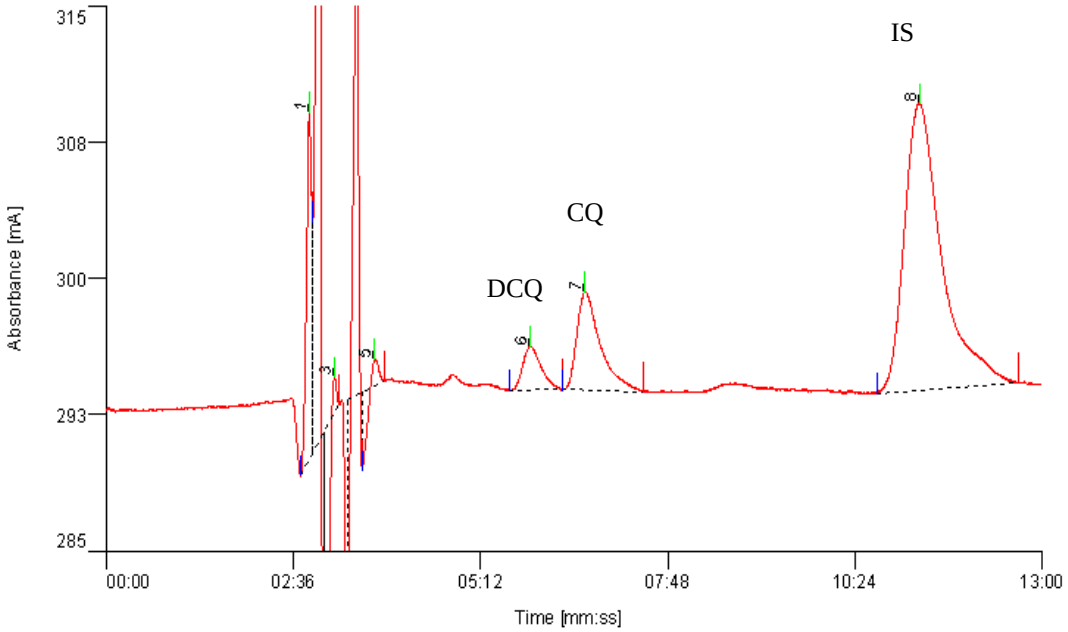

Time (min)

Figure 4.4: Chromatograms showing chloroquine (CQ) and desethyl chloroquine (DCQ) from whole blood sample collected from a patient and spotted on filter paper on day 0 (A) and day 3 (B).

4.5: Study Population

The characteristics of the 74 children who were randomly selected into the study are shown in Table 4.8. Thirty eight (51.4%) children were aged < 5 years. The geometric mean parasite density was 52,467 (95% confidence interval, 38,194 – 71,944 asexual parasite/µl of blood). Seventy eight percent (58/74) of the children were febrile at enrolment.

4.6: Pharmacokinetics parameters of all patients enrolled in the study

All the patients or guardians gave a negative history of chloroquine ingestion in the preceding two week but analysis of the pre-treatment blood sample showed substantial chloroquine levels in 43 (58.1%) of the 74 children enrolled. The mean CQ concentration was 916.86±89.79ng/ml (range, 25.6 – 2643.0ng/ml) (Table 4.8). The children were therefore divided into 4 groups for the analysis of the data; all the 74 children that were enrolled into the study; 48 children with delay in parasite clearance, 43 children with pre-dosing detectable chloroquine concentration in their blood and 31 children with no chloroquine in the pre-treatment blood sample.

Drug concentration before drug administration on day 2 (48h) was 1740.81±137.69ng/ml and the decline of chloroquine concentration after day 2 was mono exponential with a terminal half-life ($t_{1/2}$) of 306.3±20.99h (95% CI, 264.4 - 348.1h). Table 4.9 is the summary of pharmacokinetic parameters of all patients irrespective of pre-dosing chloroquine blood concentration at enrolment. The AUC_{0-28d} and CL were 920.6±69.6ng.h/ml and 633.6±60.2ml/h respectively.

Table 4.8: Baseline clinical and demographic characteristics of seventy-four children with acute uncomplicated malaria treated with standard doses of chloroquine.

Parameters	Mean±SD	95% CI
Number enrolled	74	-
Age (y)	5.2 ± 2.9	4.5 - 5.9
Range	0.5 - 12.0	-
No with age < 5 y	38 (51.4%)	-
Gender (M:F)	38:36	-
Weight (kg)	16.8 ± 6.5	15.2 - 18.3
Range	5.0 - 42.0	-
Axillary Temperature (^{O}C)	38.4 ± 1.1	38.1 – 38.6
Range	36.3 - 40.5	-
No. with Fever	58 (78.4%)	-
Haematocrit (%)	30.6±5.9	29.2 – 32.1
Range	20.0 – 46.0	-
No. with PCV < 30%	27 (36.5%)	-
Parasitaemia/µl (GM)	52,467	38,194 – 71,944
Range	2,156 - 404,666	-
CQ concentration (ng/ml) on day 0	916.8±89.7* (n=43)	735.6 – 1098.0
Range	25.6 – 2643.0	

Fever, temperature ≥ 37.5^{O}C; * Mean±s.e.m; CI, confidence interval; GM, geometric mean

Table 4.9: Pharmacokinetic parameters of chloroquine determined in children with falciparum malaria and treated standard doses of chloroquine.

Parameters	Mean ±s.e.m
$t_{1/2}$ (h)	306.3 ± 20.9
95% CI	264.4 – 348.1
Range	97.2 – 958.3
AUC_{0-28d} (ng.h/ml)	920.6 ± 69.6
95% CI	781.9 – 1059.3
Range	185.8 – 3509.2
CL (mL/h)	633.6 ± 60.2
95% CI	513.6 – 753.5
Range	69.0 – 2579.0

Cl= clearance, AUC_{0-28d} = area under concentration-time curve from day 0 -28, $t_{1/2}$= half-life

4.7: Comparison of blood chloroquine concentrations and pharmacokinetic parameters in children with early or delay in parasite clearance.

Figure 4.5 illustrates whole blood chloroquine concentrations versus time in those children with early parasite clearance or delay in parasite clearance. In these patients day 1 chloroquine concentrations were 1169.06±167.21 and 1958.23±213.80ng/ml, respectively. The difference between mean day 1 chloroquine concentration was not significant (P = 0.061) but higher in children with delay in parasite clearance. There was no correlation between delay in parasite and chloroquine concentration (P = 0.084, r = 0.237). Table 4.10 is a summary of the pharmacokinetic parameters of CQ in the children with early parasite clearance or delay in parasite clearance. There was no different between the pharmacokinetic parameters of chloroquine in children who had delay in parasite clearance compared to those whose parasites cleared early from the peripheral blood. Although the chloroquine half-life in children with delay in parasite clearance was higher compared to those that cleared parasite early (321.3±27.1 versus 219.6±25.3h, P = 0.065).

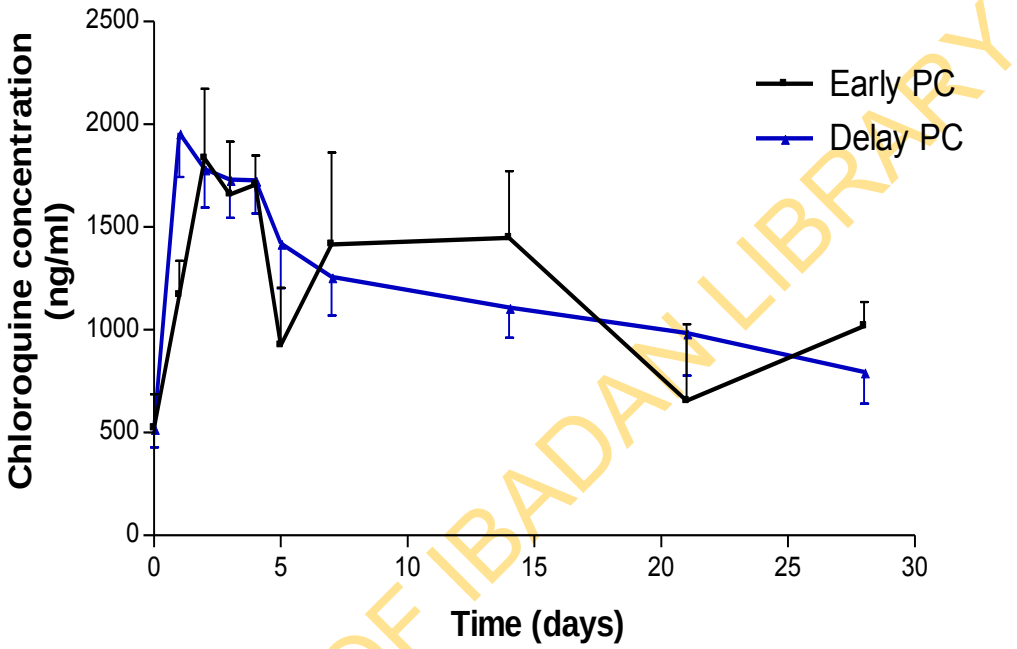

Figure 4.5: Mean whole blood concentration-time curves of chloroquine following oral administration of 25mg/kg of chloroquine base over 3 days in children infected with *Plasmodium falciparum* malaria with early or delay in parasite clearance (PC).

Table 4.10: Pharmacokinetic parameters of chloroquine in children with acute uncomplicated malaria who had delay in parasite clearance after treatment with standard doses of chloroquine

Parameters	Children with PC ≤ 2d n=13	Children with PC > 2d n= 48	P value
$t_{1/2}$ (h)	219.69 ± 25.35	321.35 ± 27.13	0.065
Range	108.0 – 379.9	97.2 – 958.3	
AUC_{0-28d} (ng/ml.h)	728.58 ± 122.99	1011.92 ± 95.17	0.150
Range	198.7 – 1577.5	185.8 – 3509.1	
CL (mL/h)	616.84 ± 92.37	612.15 ± 75.65	0.976
Range	215.0 – 1505.1	69.0 – 1952.2	

Mean±SEM, *significant value

4.8: Comparison of pharmacokinetic parameters in children with or without pretreatment chloroquine concentration.

Figure 4.6 illustrates whole blood chloroquine concentrations versus time in those children with or without CQ in their blood at enrolment. In these patients day 1 chloroquine concentrations were 1741.87±209.16 and 1640.35±226.95ng/ml, respectively. The difference between mean day 1 chloroquine concentration was not significant (P = 0.749). Day 2 concentrations were also similar: 1703.98±172.92 and 1796.90±231.34ng/ml, respectively. The difference between day 2 chloroquine concentration was not significant (P = 0.715). Table 4.11 summarizes the pharmacokinetic parameters of children with and without detectable chloroquine concentration pre-dosing. Despite presence of CQ in their blood pre-treatment there was no difference in the pharmacokinetic parameters between patients with pre-treatment CQ levels and those without detectable pre-dosing chloroquine.

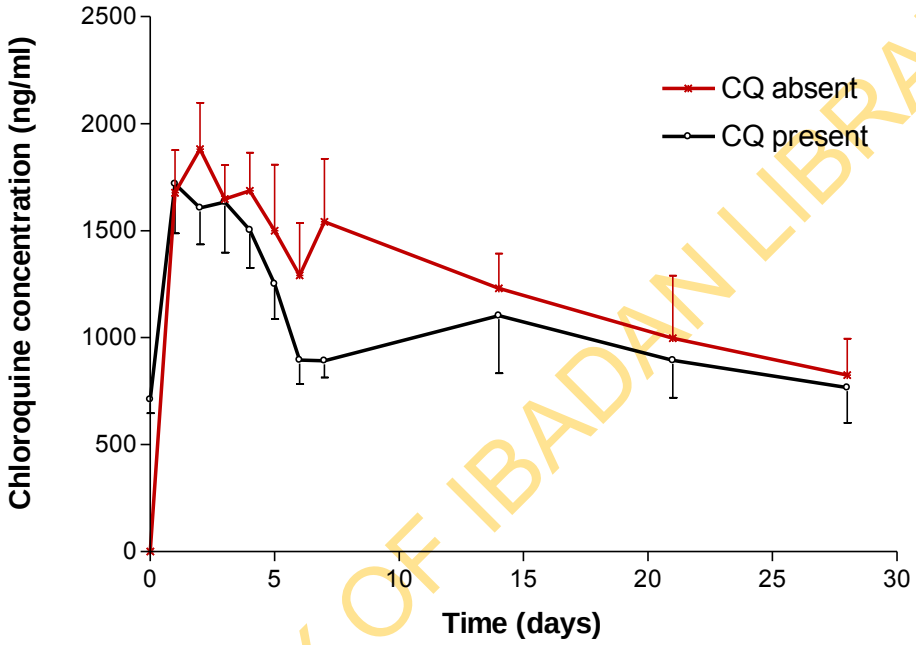

Figure 4.6: Mean whole blood concentration-time curves of chloroquine in children infected with *Plasmodium falciparum* malaria treated with oral doses of 25mg/kg chloroquine base over 3 days with or without chloroquine in their blood at enrolment.

Table 4.11: Pharmacokinetic parameters of chloroquine in children with and without pretreatment chloroquine levels following treatment with standard doses of chloroquine (n=74).

Parameters	Children without chloroquine on day 0 n=31	Children with chloroquine on day 0 n=43	P value
$t_{1/2}$ (h)	276.30 ± 23.94	327.90 ± 31.57	0.228
Range	97.2 – 666.4	108.0 – 958.3	
AUC_{0-28d} (ng/ml.h)	877.64 ± 125.86	951.60 ± 79.25	0.648
Range	185.8 – 3509.1	227.9 – 2640.0	
CL (mL/h)	666.77 ± 103.53	610.41 ± 73.09	0.604
Range	143.1 – 2579.0	69.0 – 1952.2	
Pre-dosing CQ concentration (ng/ml)	-	916.86 ± 89.79	-
Range		25.6 – 2643.0	

Mean±s.e.m, *significant value

4.9: Comparison of clinical parameters and treatment outcome in children with or without pretreatment chloroquine concentration.

Table 14.12 shows, the clinical characteristics and response to treatment in children with or without pretreatment CQ. Of the 43 or 31 children with or without CQ before treatment, 23 (53.5%) or 16 (51.6%) responded to treatment, respectively. In children with or without pretreatment CQ, 11 (25.6%), 6 (13.9%) and 3 (7.0%) or 9 (29.0%), 4 (13.0%) and 2 (6.4%) had RI, RII and RIII responses, respectively. Response to treatment in children with or without pretreatment CQ were thus similar (χ^2=0.01, P=0.9389).

4.10: Comparison of blood chloroquine concentrations in children with sensitive and resistant responses to chloroquine.

Table 4.13 is a summary of the whole blood CQ concentrations following treatment in the children with sensitive or resistant responses to chloroquine. Mean whole blood CQ concentration on day 3 was significantly higher in children with sensitive response compared with children with resistant responses (1856.7±204.7 vs 1276.3±138.2ng/ml, P = 0.011).

Table 4.12: Comparison of clinical parameters and treatment outcome in children with acute uncomplicated malaria who had or without pretreatment chloroquine concentration (n=74).

Parameters	Children without chloroquine on day 0 n= 31	Children with chloroquine on day 0 n= 43	P value
Age (y)	5.0 ± 2.6	5.3 ± 3.1	0.64
Range	1.0 – 12.0	0.5 – 12.0	
Sex (M:F)	17:14	21:22	
Weight (Kg)	15.9 ± 5.2	17.3 ± 7.2	0.38
	7.5 – 30.0	6.0 – 42.0	
FCT (d)	1.8 ± 1.1	1.9 ± 1.0	0.56
	1 – 5	1 – 5	
PCT (d)	3.3 ± 1.1	3.5 ± 1.3	0.56
	2.0 – 7.0	2.0 – 6.0	
Parasitaemia/µl (GMPD)	50,839	53,987	0.65
Range	3,125 – 313,538	2,156 – 404,666	
Response			
Sensitive	16 (51.6%)	23 (53.5%)	0.77
RI	9 (29.0%)	11 (25.6%)	
RII	4 (13.0%)	6 (13.9%)	
RIII	2 (6.4%)	3 (7.0%)	

GMPD, geometric mean parasite density, Mean±s.d

Table 4.13: Whole blood chloroquine concentrations in children with uncomplicated falciparum malaria with sensitive or resistant response after administration of 25mg/kg chloroquine base (n=74).

Time (days)	Whole blood chloroquine concentration (ng/ml)		
	Sensitive n=39	**Resistant n=35**	**P value**
0	496.47 ± 97.33*	573.22 ± 114.33	0.609
	0.0 – 2643.0**	0.0 – 2119.0	
1	1814.96 ± 219.88	1560.11 ± 212.00	0.687
	375.7 – 5192.0	74.6 – 4680.8	
2	1873.61 ± 223.98	1592.08 ± 148.76	0.312
	500.7 – 5378.1	732.9 – 4166.0	
3	1856.65 ± 204.70	1276.32 ± 138.22	0.011
	472.5 – 5776.3	135.0 – 3086.2	
7	1427.26 ± 254.75	910.20 ± 89.14	0.087
	43.7 – 5920.3	103.6 – 1892.4	
14	1214.36 ±170.27	1131.82 ± 244.01	0.789
	335.8 – 2319.6	254.5 – 1625.6	
21	1090.23 ± 259.37	757.24 ± 147.57	0.308
	77.7 – 2994.4	248.5 – 1625.9	
28	869.94 ±165.21	609.50 ± 120.68	0.328
	34.6 – 2096.3	151.6 – 1067.0	
Outcome			
Sensitive	39		
RI	-	20 (57.1%)	
RII	-	10 (28.6%)	
RIII	-	5 (14.3%)	

*Mean whole blood chloroquine concentration ±s.e.m, ** Range; RI= parasitaemia disappeara but reappears within 7 to 14 days; RII= decrease of parasitaemia but no complete clearance from peripheral blood; RIII= no pronounced decrease or increase in parasitaemia at 48hr after treatment; S= sensitive.

4.11: Comparison of chloroquine pharmacokinetic parameters in children whose infection responded or failed to respond to treatment.

Table 4.14 is a summary of the pharmacokinetic parameters of the children with sensitive or resistant responses to chloroquine. The $AUC_{0\text{-}28d}$ of chloroquine was significantly higher in children with sensitive response compared with those with resistant responses (1052.1 ± 116.3 vs 774.1± 62.9 ng/ml.h, P = 0.01). The $AUC_{0\text{-}28d}$ values in children with sensitive response was approximately 1.5 fold those with resistance response (Figure 4.7 and 4.8).

4.12: Pharmacokinetic risk factors associated with chloroquine treatment failure

Table 4.15 shows the univariate and multivariate analysis of pharmacokinetic risk factors for chloroquine treatment failure. Day 3 CQ concentration ≤ 1750ng/ml {Crude odd ratio (COR), 4.08; 95% CI, 1.13 – 14.64; P = 0.027} and $AUC_{0\text{-}28d}$ ≤ 950ng/ml.h (COR, 2.89; 95% CI, 1.05 –7.93, P = 0.037) were significantly associated with a risk of treatment failure.

However, if a P value less than 0.3 was taken ('falsed') to indicate significant difference in a univariate analysis, a terminal elimination half-life less than 220h {Adjusted odds ratio (AOR), 0.28; 95% CI, 0.08 – 0.98, P = 0.047}, and an $AUC_{0\text{-}28d}$ less than 950ng/ml.h (AOR, 4.12; 95% CI,1.09 – 15.52, P = 0.036} were independent predictors of chloroquine treatment failure (Table 4.16).

Table 4.14: Pharmacokinetic parameters of chloroquine determined in children with acute uncomplicated falciparum malaria who had sensitive or resistant responses after treatment with standard doses of chloroquine (n=74).

Parameters	Sensitive n=39	Resistant n=35	P value
$t_{1/2}$ (h)	293.8 ± 28.8	320.2 – 31.0	0.97
95% CI	235.5 – 352.0	257.2 – 383.2	
Range	97.2 – 958.3	114.0 – 875.0	
$AUC_{0\text{-}28d}$ (ng/ml.h)	1052.1 ± 116.3	774.1 ± 62.9	0.01*
95% CI	816.6 – 1285.0	646.33 – 901.8	
Range	185.8 – 3509.2	202.9 – 1890.8	
CL (mL/h)	647.9 ± 84.5	617.1 ± 86.7	0.46
95% CI	476.9 – 818.9	440.7 – 793.6	
Range	69.01 – 1952.2	107.2 – 2579.0	

Mean±s.e.m, * significant

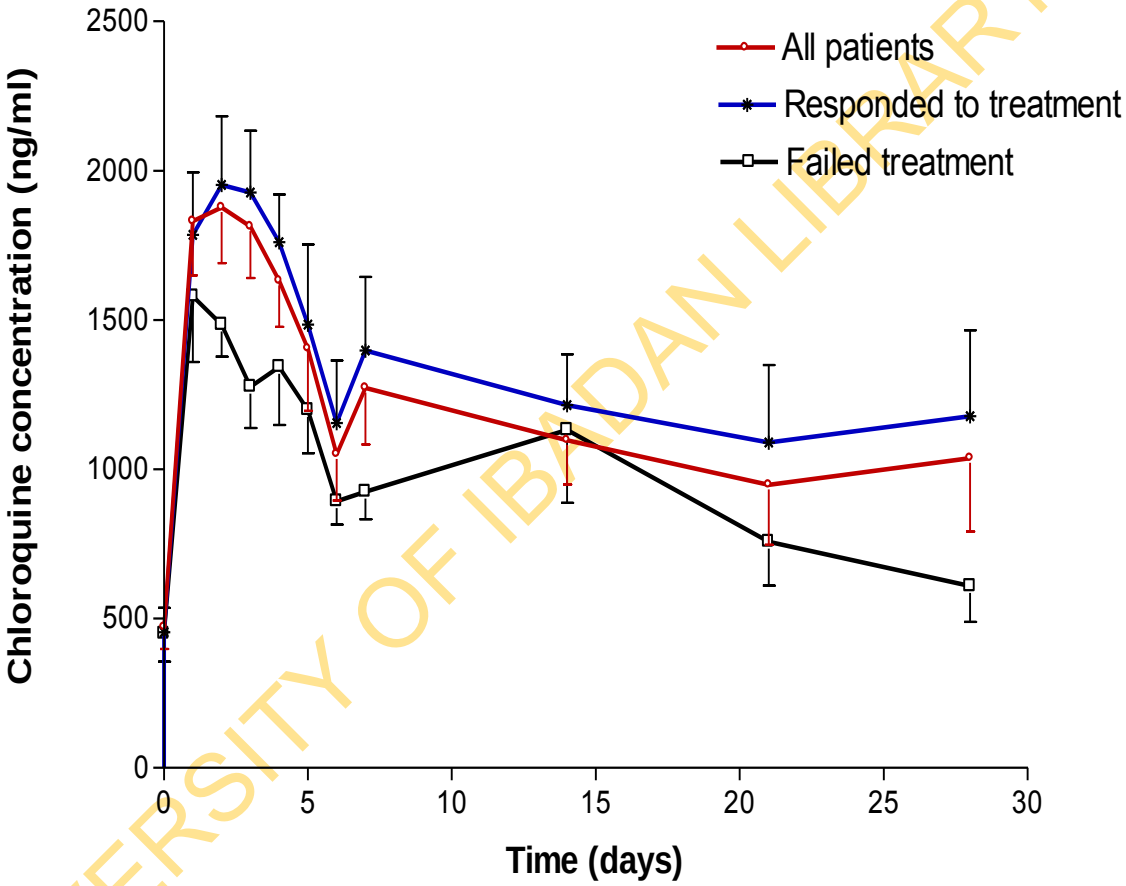

Figure 4.7: Mean concentration-time curve of chloroquine for all patients, whose infection responded or failed to respond to treatment after oral standard doses chloroquine base given over 3 days.

Table 4.15: Pharmacokinetic predictors of treatment failure in children with acute uncomplicated malaria treated with standard doses of choroquine.

	No of children		Univariate analysis		Logistic regression analysis	
	Examined	Failing treatment by day 14	Crude odds ratio (95% confidence interval)	*p* value	Adjusted odds ratio (95% CI)	*P Value*
CQ concentration (ng/mL) on day 3						
>1750	18	4	1		1	
≤1750	39	21	4.08 (1.13 – 14.64)	0.027	3.25 (0.86 – 12.23)	0.081
$t_{1/2}$ (h)						
>220	42	22	1			
≤ 220	32	13	0.62 (0.24 – 1.57)	0.300	-	-
$AUC_{0\text{-}28d}$ (ng/ml.h)						
>950	26	8	1		1	
≤ 950	48	27	2.89 (1.05 –7.93)	0.0307	3.25 (0.93 – 11.27)	0.063

Table 4.16: Pharmacokinetic risk factors of treatment failure in children with uncomplicated malaria treated with standard doses of chloroquine (P value 0.3 is considered).

	No of children		Univariate analysis		Logistic regression analysis	
	Examined	Failing treatment by day 14	Crude odds ratio (95% confidence interval)	*p* value	Adjusted odds ratio (95% CI)	*P Value*
CQ concentration (ng/mL) on day 3						
>1750	18	4	1		1	
≤1750	39	21	4.08 (1.13 – 14.64)	0.027	3.057 (0.77 - 12.13)	0.112
$t_{1/2}$ (h)						
>220	42	22	1		1	
≤ 220	32	13	0.62 (0.24 – 1.57)	0.300[@]	0.28 (0.08 – 0.98)	0.047*
$AUC_{0\text{-}28d}$ (ng/ml.h)						
>950	26	8	1		1	
≤ 950	48	27	2.89 (1.05 –7.93)	0.030	4.12 (1.09 – 15.52)	0.036*

[@]false to Multiple regression; * significant

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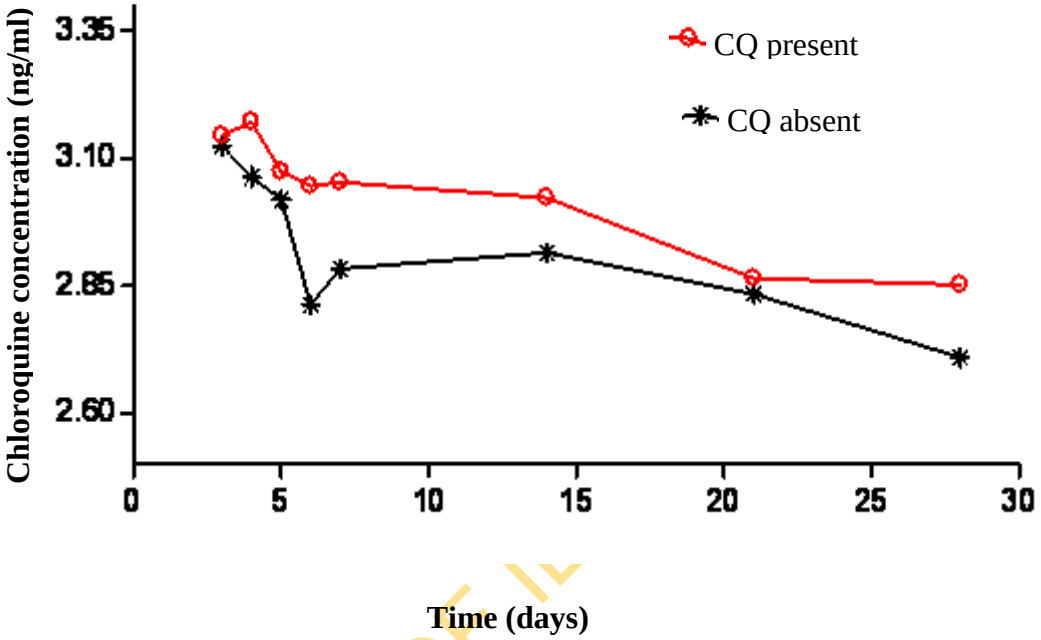

Figure 4.8: Decline of chloroquine concentration in children with or without chloroquine in their pre-treatment whole blood samples following oral administration of 25mg/kg of chloroquine base over 3 days.

Study 3: Development of a simple cost effective high performance liquid chromatography assay of sulphadoxine in whole blood spotted on filter paper for field studies.

4.13: Chromatography techniques

Standard curves from sulphadoxine spiked blood added to filter paper were linear over the concentration ranged studied. Linear regression analysis yielded correlation coefficient r > 0.99 (n = 6, Figure 4.9). Sulphadoxine peak was well resolved from the internal standard (sulizoxasole) at the calibration ranges of 0 – 60 µg/ml (Appendix Table 4.b). The retention times (t_R) of sulphadoxine and internal standard (IS) were 6.3 and 7.2min respectively (Figure 4.10). The separation chromatograms of sulphadoxine and the internal standard from spiked whole blood samples corresponded with those of blood samples obtained from a patient at time 0 before drug administration and day 3 after an oral standard single dose of sulphadoxine-pyrimethamine (SP) (25mg/kg body weight of sulphadoxine and 1.25mg/kg body weight of pyrimethamine (Figure 4.10 A, B and C respectively).

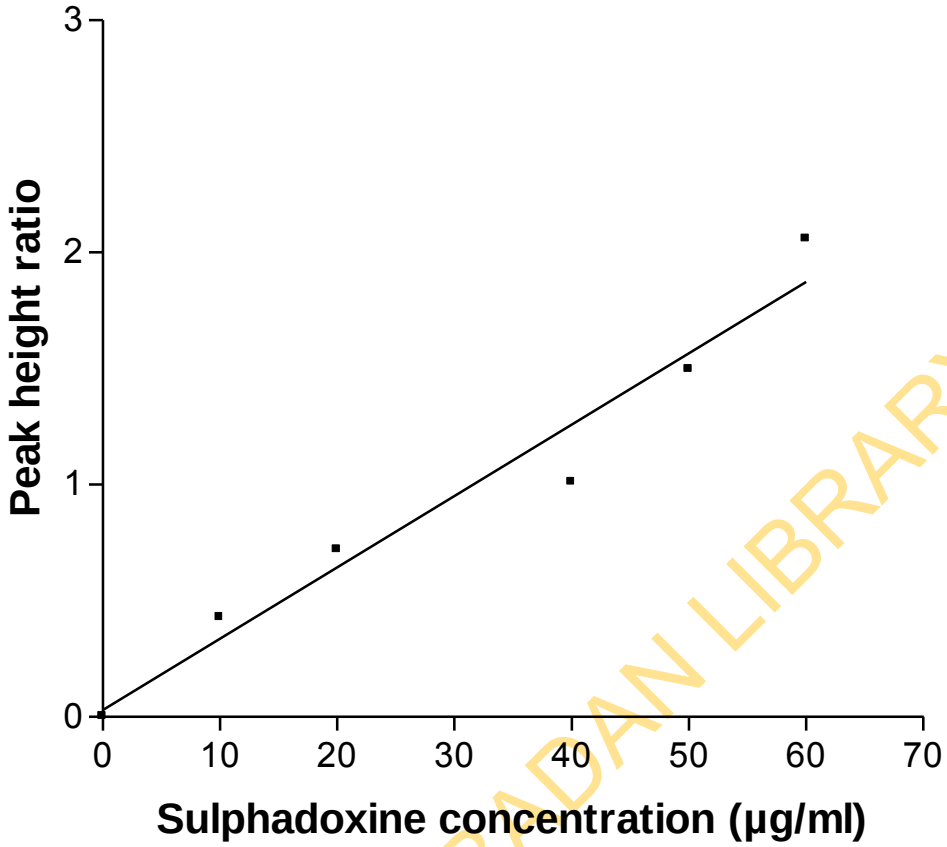

Figure 4.9: Sulphadoxine standard curve for extrapolation of sulphadoxine concentrations from unknown blood sample spotted on filter paper (r^2=0.99518).

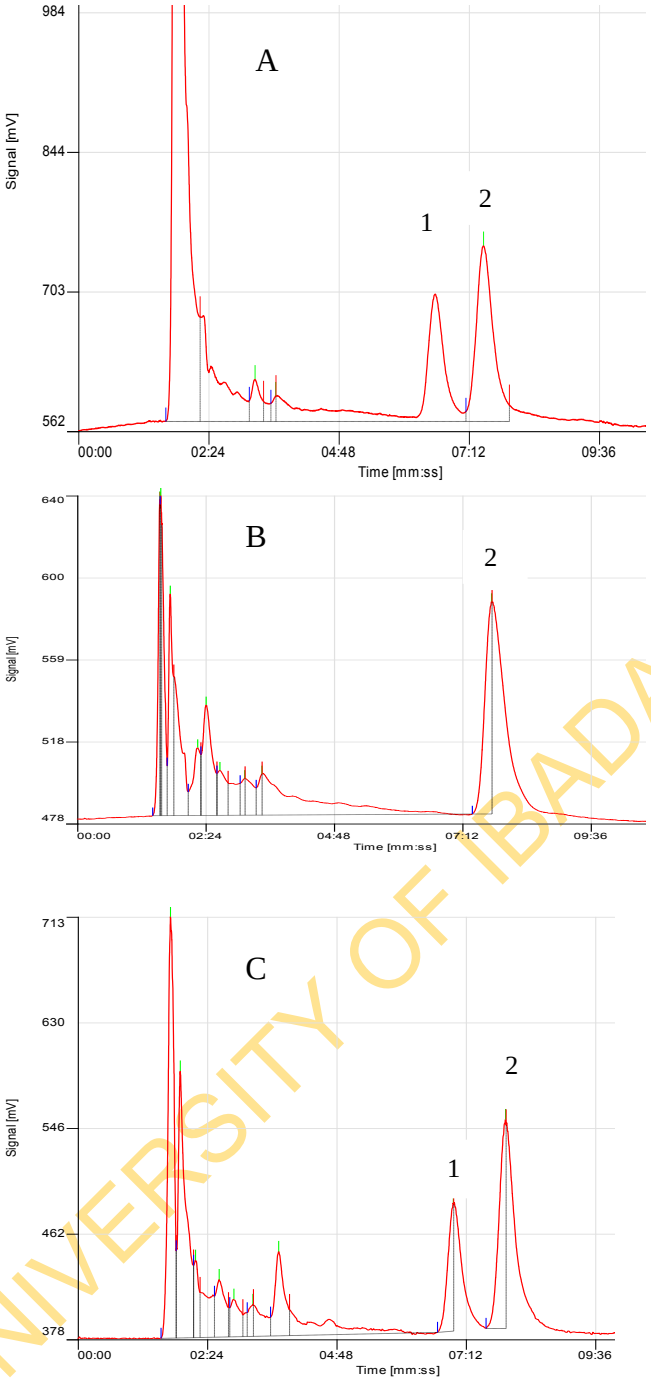

Figure 4.10: Chromatograms showing peaks after extraction of sulphadoxine (1) and internal standard (2) from drug free whole blood spiked with 60µg/ml sulphadoxine (A), day 0 (B) and day 3 (C) of sample collected from a patient who was administered with a standard dose of sulphadoxine/pyrimethamine.

4.14: Recovery, calibration curves and reproducibility

The extraction recoveries for 25µg/ml, 60µg/ml and 100µg/ml of sulphadoxine were 82.66±4.0 (n=6), 81.02±3.24 (n=5) and 85.60±1.9 (n=5) per cent respectively (Table 4.17). The intra-day recovery deviation at 60µg/ml and 100µg/ml of SDX were 3.7% and 4.6% respectively (n=5) (Table 4.18). The intra-day precision was <5.0% and inter-day accuracy ranged from 4.1 to 5.3% respectively. The limit of detection of sulphadoxine defined as a concentration giving a peak four times the baseline noise in all biological fluids was 120ng/mL at 0.05 absorbance units full scale (aufs).

Table 4.17: Percentage recovery of sulphadoxine from whole blood samples collected on filter paper.

	Concentration (µg/mL)	% Recovery ±S.D	N
Whole blood	25	82.6±4.0	6
	60	81.0±3.2	5
	100	85.6±1.9	5

SD= standard deviation

Table 4.18: Precision of analytical method for sulphadoxine determination using whole blood spotted on filter paper.

	Concentration (µg/mL)	C.V (%)	n
Intra-Assay	Sulphadoxine		
	60	3.7±3.0	5
	100	4.6±2.5	5
Inter-Assay	Sulphadoxine		
	10	6.0±2.0	5
	100	4.1±2.0	6

CV= coefficient of variation

4.15: Interference

There was no interference from endogenous compounds in the biological sample. There was no interference with the peaks of sulizoxasole (IS) and other commonly used antimalarial, analgesic and anti-infective drugs.

4.16: Clinical application

The concentration-time curve in whole blood of a patient who was administered with a standard single oral dose of sulphadoxine-pyrimethamine (SP) (25mg/kg body weight of sulphadoxine and 1.25mg/kg body weight of pyrimethamine i.e 500mg of sulphadoxine and 25mg of pyrimethamine) is shown in Figure 4.11. The pharmacokinetic parameters of SDX in the individual are shown in Table 4.19. Peak blood concentration of 212.02µg/ml was reached after day 1. The calculated elimination half-life was 3.50d while the areas under the concentration time curve ($AUC_{0\text{-}28d}$) were 884.84µg/ml.d. The profile show the applicability of the method for measuring SDX in whole blood dried on filter. The pharmacokinetic result agrees with previous reports (Dzinjalamala *et al*., 2005). The method was not sensitive to detect pyrimethamine, although pyrimethamine peak was detected and separated during the standard calibration preparations. This insensitivity may be due to the fact that amount of pyrimethamine in tablet was small (1:20 of sulphadoxine) and only a small volume of blood used for the analysis.

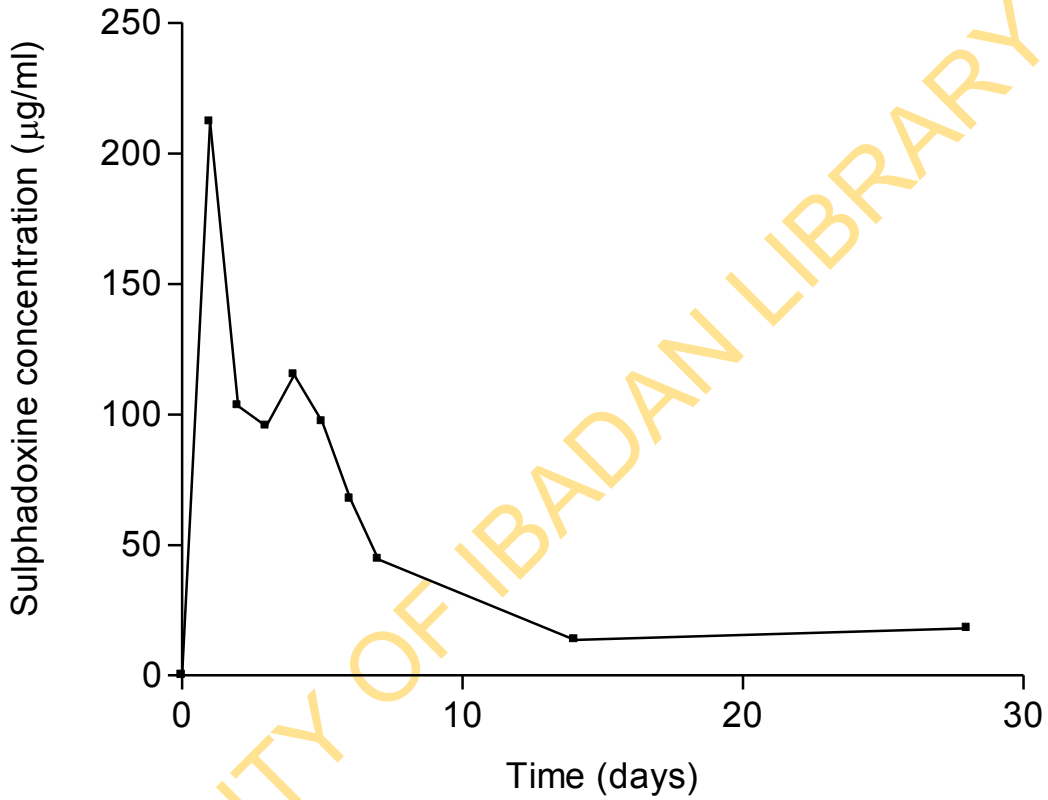

Figure 4.11: Concentration–time curve for sulphadoxine in whole blood following single oral dose of sulphadoxine-pyrimethamine (25mg/kg body weight of sulphadoxine and 1.25mg/kg body weight of pyrimethamine).

Table 4.19: Demographic and pharmacokinetic parameters of a patient who received a standard dose of sulphadoxine-pyrimethamine.

Parameters	**Mean**
Age (year)	6.0
Weight (Kg)	16.0
$t_{1/2}$ (d)	3.50
T_{max} (d)	1.00
C_{max} (µg/ml)	212.02
$AUC_{0\text{-}28d}$ (µg/ml.d)	884.84
Cl (ml/d)	524.50

$AUC_{0\text{-}28}$, area under concentration time –curve from time 0 to day 28, Cmax, peak blood concentration; Tmax, time to peak blood concentration; $t_{1/2}$ elimination half-life; Cl, clearance.

Study 4: Pharmacokinetic disposition of sulphadoxine in children with uncomplicated *P. falciparum* malaria treated with standard dose of sulphadoxine-pyrimethamine.

4.17: Study population

A total of 74 patients with acute uncomplicated malaria were randomly selected into the study. The clinical and demographic parameters of the children are shown in Table 4.20. The geometric mean parasite density was 48,953 (Range, 2011- 461,333 asexual parasite/µl of blood). The mean age of the children was 5.07±3.02 years and their mean weight was 16.55±7.7kg.

Table 4.20: Baseline clinical and demographic characteristics of children with acute uncomplicated falciparum malaria treated with standard dose of sulphadoxine-pyrimethamine (n=74).

Parameters	Mean±SD	95% CI
Number enrolled	74	-
Age (y)	5.2 ± 3.0	4.5 - 5.9
Range	0.5 - 12.0	-
No with age < 5year	38 (51.4%)	-
Gender (M:F)	35:39	-
Weight (kg)	16.9 ± 7.6	15.1 - 18.7
Range	4.0 – 38.0	-
Axillary Temperature (^{O}C)	38.2 ± 1.0	37.2 – 38.4
Range	36.1 - 40.5	-
No. with fever	57 (77.0%)	-
Haematocrit (%)	30.7±4.0	29.1 – 32.3
Range	25.0 – 38.0	-
No. with PCV < 30%	27 (36.5%)	-
Parasitaemia/µl (GM)	48,953	
Range	2011- 461,333	-
SDX Concentration (µg/ml) on day 0	8.9 ± 3.5* (n=13)	
Range	2.6 – 168.3	

Fever, temperature >37.5^{O}C; * Mean±s.e.m; CI, confidence interval; GM, geometric mean

4.18: Pharmacokinetic parameters of sulphadoxine in children with uncomplicated *falciparum* malaria after administration of standard dose of sulphadoxine-pyrimethamine.

Thirteen patients (17.6%) had pretreatment levels of SDX and their data were excluded from the final analysis. The mean SDX concentration was 8.9±3.5µg/ml (range, 2.6 – 168.3µg/ml) (Table 4.20). The patients were therefore divided into 4 groups for the analysis of the data; the 74 children that were enrolled into the study; 45 with delay in parasite clearance; 13 with detectable pre-treatment SDX concentration and 61 children without SDX before treatment.

Table 4.21 is the summary of the pharmacokinetic parameters of all the patients irrespective of pre-treatment SDX blood concentration at enrolment. The mean t_{max} and elimination half-life of SDX in capillary whole blood were 2.1±0.1 and 5.5±0.3d. The peak whole blood concentration (C_{max}) ranged from 25.5 to 745.69µg/ml with mean of 214.9±16.9µg/ml. The $AUC_{0\text{-}28d}$ varied between 91.19 and 3768.62µg/ml.d (mean 1252.9±106.1µg/ml.d). The clearance was 437.1±39.9ml/d (ranged, 86.2 – 1555.3).

Table 4.21: Pharmacokinetic parameters of sulphadoxine in seventy-four children treated with standard dose of sulphadoxine-pyrimethamine.

Parameters	Mean ±s.e.m
C_{max} (µg/ml)	214.9 ± 16.9
Range	25.5 – 745.69
T_{max} (d)	2.1 ± 0.1
Range	1 – 5
$t_{1/2}$ (d)	5.5 ± 0.3
Range	1.1 – 17.0
$AUC_{0\text{-}28d}$ (µg/ml.d)	1252.9 ± 106.1
Range	91.1 – 3768.6
CL (mL/d)	437.1 ± 39.9
Range	86.2 – 1555.5
Day 3 SDX concentration (µg/ml)	184.9 ± 20.0
Range	19.7 – 856.9
Day 7 SDX concentration (µg/ml)	85.0 ± 11.2
Range	3.0 – 368.9
Day 14 SDX concentration (µg/ml)	43.9 ± 6.6
Range	3.7 – 151.6

4.19: Comparison of blood sulphadoxine concentrations and pharmacokinetic parameters in children with early parasite clearance or delay in parasite clearance.

Figure 4.12 illustrates whole blood sulphadoxine concentrations versus time in those children with early parasite clearance or delay in parasite clearance. In these patients day one sulphadoxine concentrations were 127.0±19.5 or 215.2±25.5 µg/ml, respectively. The difference between mean day 1 SDX concentration was significantly (P = 0.049) higher in children with delay in parasite clearance. Table 4.22 is a summary of the pharmacokinetic parameters of SDX in the children with early parasite clearance or delay in parasite clearance. There was no difference between the pharmacokinetic parameters of sulphadoxine in children who had delay in parasite clearance compared to those that their parasites cleared early from the peripheral blood. Afterwards, the concentrations of SDX in children who cleared parasite early were higher, although not statistically significant. The mean maximum concentration of SDX (C_{max}) obtained in children who cleared parasite early was high but not significant.

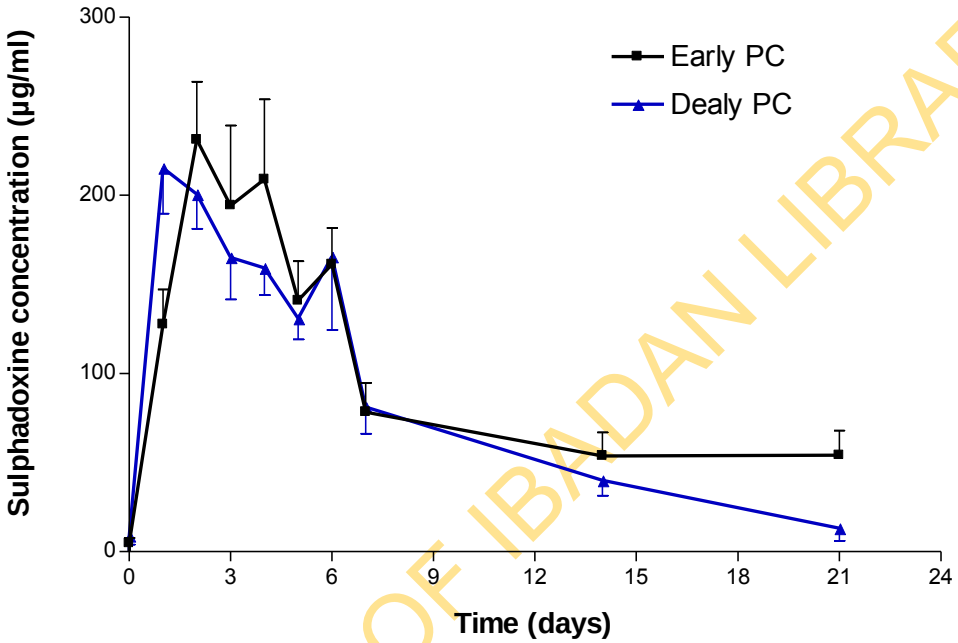

Figure 4.12: Mean whole blood concentration-time curves of sulphadoxine in children infected with *Plasmodium falciparum* malaria with early or delay in parasite clearance.

Table 4.22: Comparison of pharmacokinetic disposition of sulphadoxine in children with and without delay in parasite clearance (n=65)

Parameters	Children with PC ≤ 2d (n=20)	Children with PC > 2d (n= 45)	P value
t_{max} (d)	1.95 ± 0.23	2.09 ± 0.15	0.611
Range	1.0 – 4.0	1.0 – 4.0	
C_{max} (µg/ml)	218.49 ± 38.65	194.10 ± 18.64	0.522
Range	137.5 – 299.4	156.4 – 231.7	
$t_{1/2}$ (d)	5.01 ± 0.53	5.59 ± 0.55	0.524
Range	1.4 – 11.0	1.16 – 17.0	
$AUC_{0\text{-}28d}$ (µg/ml.d)	1102.45 ± 187.78	1011.92 ± 95.17	0.150
Range	91.1 – 2568.9	185.8 – 3509.1	
CL (mL/d)	616.84 ± 92.37	612.15 ± 75.65	0.976
Range	215.0 – 1505.1	69.0 – 1952.2	

Mean±s.e.m, *significant value

4.20: Comparison of disposition of sulphadoxine in children with and without detectable blood levels of sulphadoxine at enrolment before treatment.

Figure 4.13 illustrates whole blood sulphadoxine concentrations versus time in those children with or without SDX in their blood at enrolment. The mean concentration of SDX at presentation in 13 patients who had detectable SDX level before treatment was 8.90±3.5µg/ml (range 2.99 - 168.3µg/ml). In children with or without SDX in their blood at enrolment, day 1 SDX concentrations were 188.94±22.2 or 274.04±71.4µg/ml (P=0.225) respectively. Day 2 concentrations were also similar; 211.57±18.1 or 261.69±45.4µg/ml (P=0.343) between children with or without SDX in their blood at enrolment. Comparison of disposition of SDX in children with pretreatment SDX levels and those without SDX showed a similar disposition profile; C_{max} (P=0.325), T_{max} (p=0.128), Cl (P=0.377), day 3 concentration (P=0.343), day 7 concentration (P=0.249), and day 14 SDX concentration (P=0.245). Table 4.23 summarizes the pharmacokinetic parameters of children with and without detectable sulphadoxine concentration pre-treatment. However, a trend towards a shorter mean elimination half-life (3.91±0.40d versus 5.85.69±0.44d, P=0.063) and a higher AUC_{0-28d} (1660.33±172.59 versus 1161.62±121.39 µg/ml.d, P=0.069) was observed in patients with pre-treatment SDX levels.

Table 4.23: Pharmacokinetic parameters of sulphadoxine in children with and without sulphadoxine in pretreatment whole blood samples.

Parameters	Children without sulphadoxine on day 0 (n=61)	Children with sulphadoxine on day 0 (n=13)	P values
$t_{1/2}$ (d)	5.85±0.44	3.91±0.40	0.063
Range	1.16-17.01	2.31-7.04	
C_{max} (µg/mL)	207.75±19.31	254.10±27.24	0.325
Range	25.5-856.9	112.6-413.6	
t_{max} (d)	2.19±0.14	1.66±0.28	0.128
Range	1.0-5.0	0.0-3.0	
AUC_{0-28d} ($\mu gmL^{-1}.d$)	1161.62±121.39	1660.33±172.59	0.069
Range	91.1-3768.6	221.6-2540.9	
Cl (mL/d)	454.26±47.19	363.16±59.03	0.377
Range	86.2-1555.3	96.0-936.58	
Geometric mean (parasite/µL blood)	47081.70	59293.28	0.925
Range	2011 -461333	6973-33684	
Day 0 Conc. (µg/ml)	-	8.90±3.54	-
Range		2.62-168.32	
Day 3 Conc. (µg/ml)	175.81±22.59	225.17±42.67	0.343
Range	19.71-856.98	60.45-636.54	
Day 7 Conc.(µg/ml)	78.86±13.07	112.74±47.44	0.249
Range	19.71-856.98	66.00-205.32	
Response			
ACPR	50 (82.0%)	6 (46.2%)	
ETF	2 (3.2%)	2 (15.4%)	
LCF	9 (14.8%)	5 (38.4%)	

Values are in Mean±s.e.m

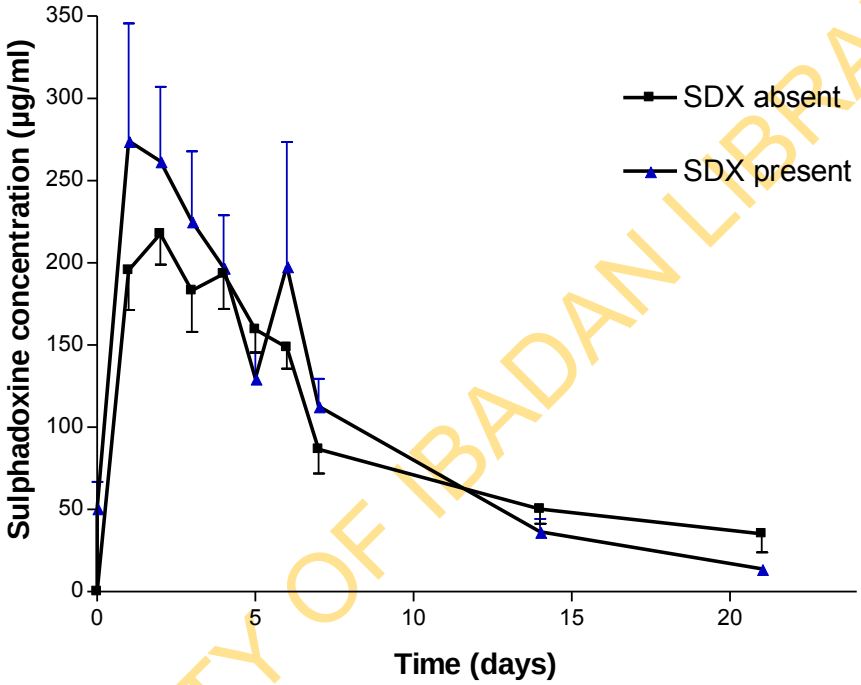

Figure 4.13: Mean whole blood sulphadoxine concentration-time curves in children with *Plasmodium falciparum* malaria with or without sulphadoxine in their blood at enrolment.

4.21: Pharmacokinetics of sulphadoxine in 61 children without pretreatment sulphadoxine at enrolment.

The characteristics and demographic parameters of the 61 children without pretreatment SDX are shown in Table 4.24. Thirty one (51.6%) children were aged < 5 years. The geometric mean parasite density was 472,467 (range 2011 - 461,333 asexual parasite/µl). Infection in 50 (81.9%) of the patients exhibited adequate clinical response (ACPR), whereas 3.3% (2/61) of the patient exhibited early treatment failure (ETF) and 14.8% (9/61) had late parasitological failure (LPF).

Table 4.24: Clinical and laboratory data at enrolment of children with acute, uncomplicated falciparum malaria treated with standard dose of sulphadoxine-pyrimethamine without pre-treatment sulphadoxine levels.

Parameters	**Sulphadoxine**
Number enrolled	61
Gender (no. of males: no of females)	31:30
Age (yr)	5.16±3.0
Range	0.5-12.0
Weight (kg)	16.04±6.9
Range	4.0-38.0
Axillary Temperature (^{O}C)	38.16±1.1
Range	36.1-40.5
Geometric Mean (Asexual parasite per µl of blood)	47,081
Range	2,011-461,333
Cure rate on day 14 (%)	
ACPR	81.9 (50/61)
ETF	3.3 (2/61)
LPF	14.8 (9/61)

Values are in Mean±Standard deviation,

4.22: Comparison of blood sulphadoxine concentrations in children with sensitive and resistant responses to sulphadoxine-pyrimethamine.

Table 4.25 is a summary of the whole blood SDX concentrations following treatment in children whose infection were sensitive or resistant to sulphadoxine-pyrimethamine. There was no difference in whole blood sulphadoxine concentration in children whose infection were sensitive or resistant to treatment. Figure 4.14 shows the mean concentration-time curves for the disposition of SDX in children whose infections were sensitive or resistant to SP treatment. There was inter-individual variation in the achieved sulphadoxine concentration in the children.

4.23: Comparison of disposition of sulphadoxine in children whose infection responded or failed to respond to standard dose of sulphadoxine-pyrimethamine.

Table 4.26 is a summary of the pharmacokinetic parameters of the children whose infection were sensitive or resistant to sulphadoxine-pyrimethamine. The $t_{1/2}$ of sulphadoxine in children whose infections were sensitive or resistant to SP was 5.98±0.5 or 5.26±1.0d respectively. The C_{max} of SDX ranged from 42.1 to 745.69µg/mL and 25.5 to 565.12µg/mL in patients whose infections were sensitive and resistant to sulphadoxine-pyrimethamine. There was no difference between the mean peak sulphadoxine concentration (C_{max}) in patients who failed to respond to treatment (163.09±30.3µg/mL) and those whose infection were sensitive (217.78±22.5µg/mL) to treatment.

Table 4.25: Whole blood sulphadoxine concentration in children with uncomplicated falciparum malaria with sensitive or resistant response after oral administration of a standard dose of sulphadoxine-pyrimethamine (n=61).

Time (days)	Whole blood sulphadoxine concentration (µg/ml)		
	Sensitive (n=50)	**Resistant (n=11)**	**P value**
0	0.0	0.0	-
1	191.03 ± 23.43*	171.65 ± 81.15	0.791
	22.3 – 565.1**	62.1 – 413.1	
2	215.60 ± 20.37	192.72 ± 42.73	0.644
	82.3 – 421.3	49.5 – 270.9	
3	179.06 ± 25.83	157.53 ± 38.23	0.737
	19.7 – 856.9	39.2 – 347.9	
7	84.24 ± 14.92	51.95 ± 23.32	0.365
	3.0 – 368.9	3.7 – 155.5	
14	50.21 ± 8.85	14.59 ± 5.95	0.151
	4.7 – 151.6	3.7 – 24.1	
21	35.09 ± 11.17	8.40 ± 6.25	0.310
	2.6 – 81.8	2.16 – 14.66	

* Mean± sem whole blood SDX concentration, ** Range

Table 4.26: Pharmacokinetic parameters of sulphadoxine in children without pre-treatment sulphadoxine with uncomplicated falciparum malaria who had sensitive or resistant responses to oral administration of sulphadoxine-pyrimethamine (n=61).

Parameters	Sensitive (n=50)	Resistant (n=11)	P value
C_{max} (µg/ml)	217.78 ± 22.5	163.09 ± 30.35	0.277
Range	42.1 – 745.6	25.5 – 347.9	
T_{max} (d)	2.10 ± 0.14	2.63 ± 0.41	0.148
Range	1.0-5.0	1.0 – 5.0	
$t_{1/2}$ (d)	5.98 ± 0.50	5.26 ± 1.01	0.540
Range	1.1 – 17.0	1.2 – 13.3	
$AUC_{0\text{-}28d}$ (µg/ml.d)	1196.18 ± 131.83	1013.93 ± 314.00	0.561
Range	91.1 – 3679.1	125.4 – 3768.6	
CL (mL/d)	437.45 ± 49.16	531.61 ± 140.81	0.450
Range	86.2 – 1356.1	98.3 – 1555.3	
Parasite density (GM)	47260	46311	0.830
Range	2011 – 461,333	2574 – 231,333	

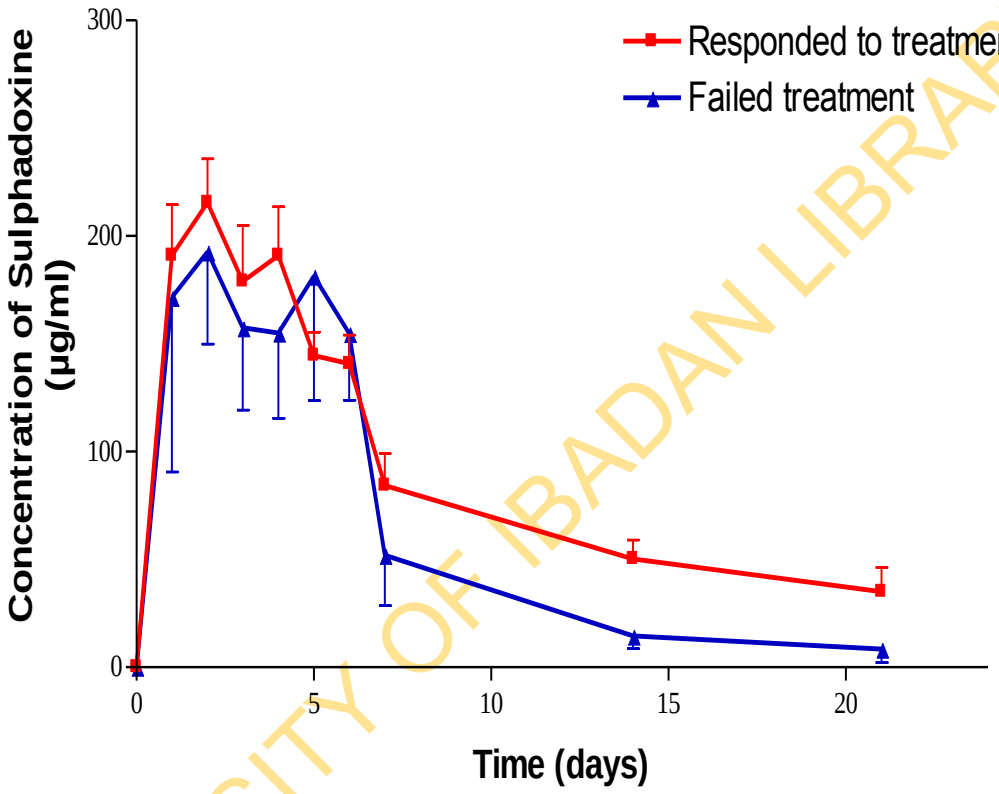

Figure 4.14.: Mean whole blood concentration-time profile of sulphadoxine in children whose infection responded and whose infection failed to respond to treatment after oral administration of standard dose of sulphadoxine-pyrimethamine (25mg/kg S-1.25mg/kg P).

4.24: Relationship between age and sulphadoxine pharmacokinetic parameters in children treated with standard oral dose of sulphadoxine-pyrimethamine.

Figure 4.15 shows the mean concentration-time profile of sulphadoxine in children < 5 years and ≥ 5years who took oral dose of sulphadoxine-pyrimethamine for the treatment of uncomplicated malaria during infection. The mean SDX concentration on day 1 (126.9±30.5 versus 235.9±27.9µg/ml, P=0.015), day 3 (98.1±15.9 versus 245.1±35.7µg/ml, P=0.001) and day 7 (54.1±10.5 versus 122.5±27.7µg/ml, P=0.01) were significantly higher in children older than 5 years compared to those less than 5years of age (Table 4.27).

Table 4.28 describes the effect of age on disposition of sulphadoxine in children infected with *Plasmodium falciparum.* A significant difference in disposition of SDX was observed between children younger and older than 5 years of age. The C_{max} of SDX in ≥5 years age group (295.7 ± 28.7 µg/ml) was significantly ($p<0.001$) higher than age group of children <5years (125.4 ± 15.2µg/ml). The time (t_{max}) taken to reach this peak concentration were similar (2.0 ± 0.18d versus 2.4± 0.2d, p=0.160). The extent of exposure ($AUC_{0\text{-}28d}$) of SDX obtained in children ≥5 years of age (1562.9 ± 202.3µg/ml.d) was significantly higher ($p<0.001$) compared to the age group less than 5 years (812.0 ± 112.7µg/ml.d) respectively. The values of Cmax and AUC in older children were twice as high as those in younger children. In contrast, the mean clearance of SDX was significantly lower in older children (334.3 ± 45.8 versus 574.1±76.8 ml/d, P=0.010). However, response to treatment in the two age

groups was not significantly different (P=0.352) though a higher proportion of children below 5 years failed treatment with SP.

Table 4.27: Whole blood sulphadoxine concentration in children with uncomplicated falciparum malaria with sensitive or resistant responses after administration of standard oral dose sulphadoxine-pyrimethamine (n=61).

	Whole blood sulphadoxine concentration (µg/ml)		
Time (days)	**<5 years (n= 31)**	**≥ 5 years (n=30)**	**P value**
0	0.0	0.0	-
1	126.99 ± 13.09*	235.93 ± 27.92	0.015
	36.1 – 413.1**	98.7 – 565.1	
2	182.57 ± 40.75	225.36 ± 20.39	0.344
	49.1 – 312.1	103.2 – 421.3	
3	98.10 ± 15.94	245.19 ± 35.79	0.001
	22.1 – 347.9	191.7 – 856.9	
7	54.17 ± 10.59	122.54 ± 27.70	0.010
	3.0 – 217.7	17.3 – 368.9	
14	38.64 ± 9.01	57.61 ± 15.52	0.268
	3.7 – 115.3	12.3 – 151.6	
21	28.28 ± 13.62	30.67 ± 11.69	0.930
	14.6 – 41.9	10.2– 81.6	

*Mean±sem whole blood SDX concentration, ** Range

Table 4.28: Relationship between age and pharmacokinetic parameters of sulphadoxine in children with uncomplicated falciparum malaria treated with standard sulphadoxine-pyrimethamine (n=61).

Parameters	**<5 year (n=31)**	**≥5 Year (n=30)**	**P value**
C_{max} (µg/ml)	125.45 ± 15.22	295.72 ± 28.72	0.000
Range	25.5 – 347.9	98.0 – 745.6	
T_{max} (d)	2.00 ± 0.18	2.40 ± 0.21	0.160
Range	1.0-4.0	1.0 – 5.0	
$t_{1/2}$ (d)	5.61 ± 0.58	6.10 ± 0.70	0.593
Range	1.2 – 13.8	1.1 – 17.0	
$AUC_{0\text{-}28d}$ (µg/ml.d)	812.09 ± 112.77	1562.93 ± 202.3	0.001*
Range	91.1 – 2194.5	222.9 – 3768.6	
Cl (mL/d)	574.14 ± 76.84	334.39 ± 45.88	0.010*
Range	86.2 – 1555.3	86.6 – 867.9	
Parasite density (GM)	38540	57133	0.891
Range	2011 – 461333	2574 – 231,333	
Parasite reduction ratio (PRR)	2.29 ± 0.98	3.32 ± 0.62	0.001*
Response			
ACPR	24 (77.4%)	26 (86.7%)	
ETF	1 (3.2%)	1 (3.3%)	
LPF	6 (19.4%)	3 (10.0%)	0.352

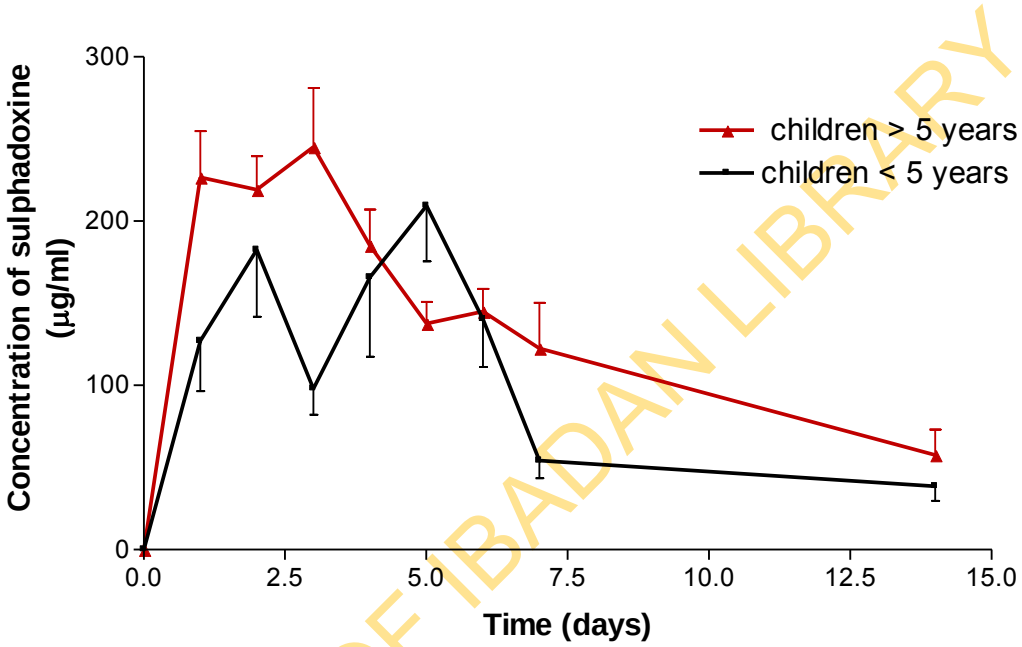

Figure 4.15: Mean concentration-time curve of sulphadoxine in capillary whole blood of children younger than 5 years or older than 5 years after oral administration of standard dose of sulphadoxine-pyrimethamine.

Study 5: Evaluation of the use of saliva for therapeutic drug monitoring in children with uncomplicated *Plasmodium falciparum* malaria treated with amodiaquine-artesuante.

Amodiaquine (AQ) and desethyl amodiaquine (DEAQ) were well resolved in the analysis using the modified method of Gitau and others (Gitau *et al*., 2004). There was a good linearity (r^2 = 0.9907 plasma; Figure 4.16 and appendix Table 4.c; and saliva, r^2=0.9838, Figure 4.18 and appendix Table 4.d) in the standard curve obtained for desethyl amodiaquine in plasma and saliva. In addition, the recoveries for amodiaquine and desethylamodiaquine over the concentration range of 100 ng/ml to 600 ng/ml were between 69.7 and 79.4% for plasma and saliva, respectively. Coefficient of variation within samples were 5.7% - 2.0%, and between samples 7.2% - 3.5%, for concentrations of 100 ng/ml to 800 ng/ml, respectively. The compound eluted from the system in the order of quinidine, desethylamodiaquine and amodiaquine. Figure 4.18 shows the chromatograms for IS, DEAQ and AQ in plasma and saliva. There was no interference with other drugs (e.g. paracetamol and other commonly used drug such as antimalarial, analgesic and anti-infective drugs) with the peaks obtained (Figure 4.18). The lower limit of detection for desethyl amodiaquine was 15ng/ml at 0.05 absorbance units' full scale (aufs). The retention time for IS, DEAQ and AQ were 5.5, 9.5 and 11.5 minutes respectively.

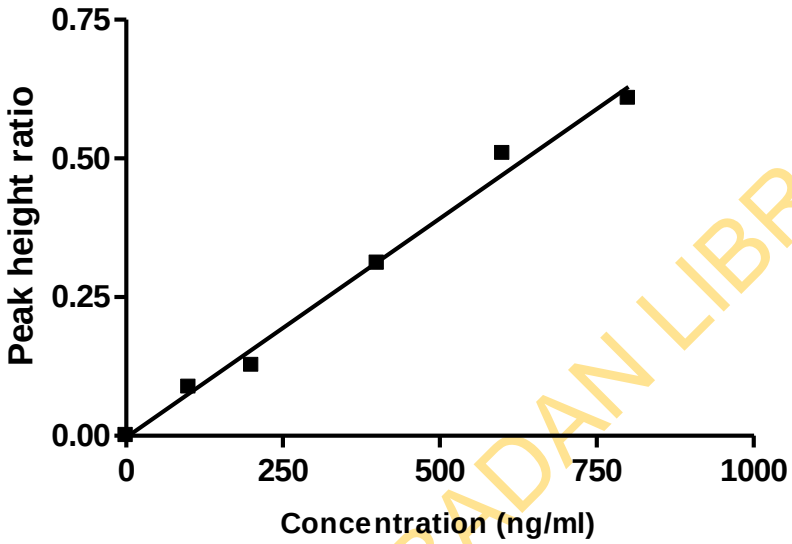

Figure 4.16: Standard curve of desethyl amodiaquine for extrapolation of unknown concentrations of DEAQ in plasma (r^2 = 0.9907).

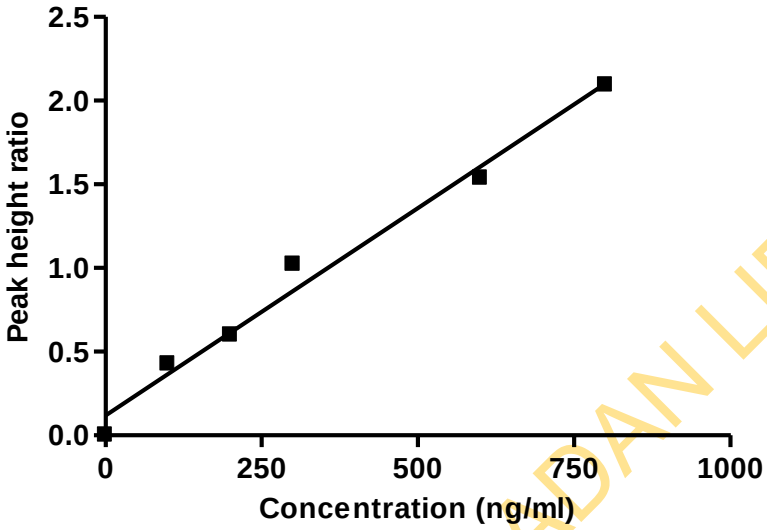

Figure 4.17: Standard curve of desethyl amodiaquine for extrapolation of unknown concentrations of desethyl amodiaquine in saliva (r^2 = 0.9838).

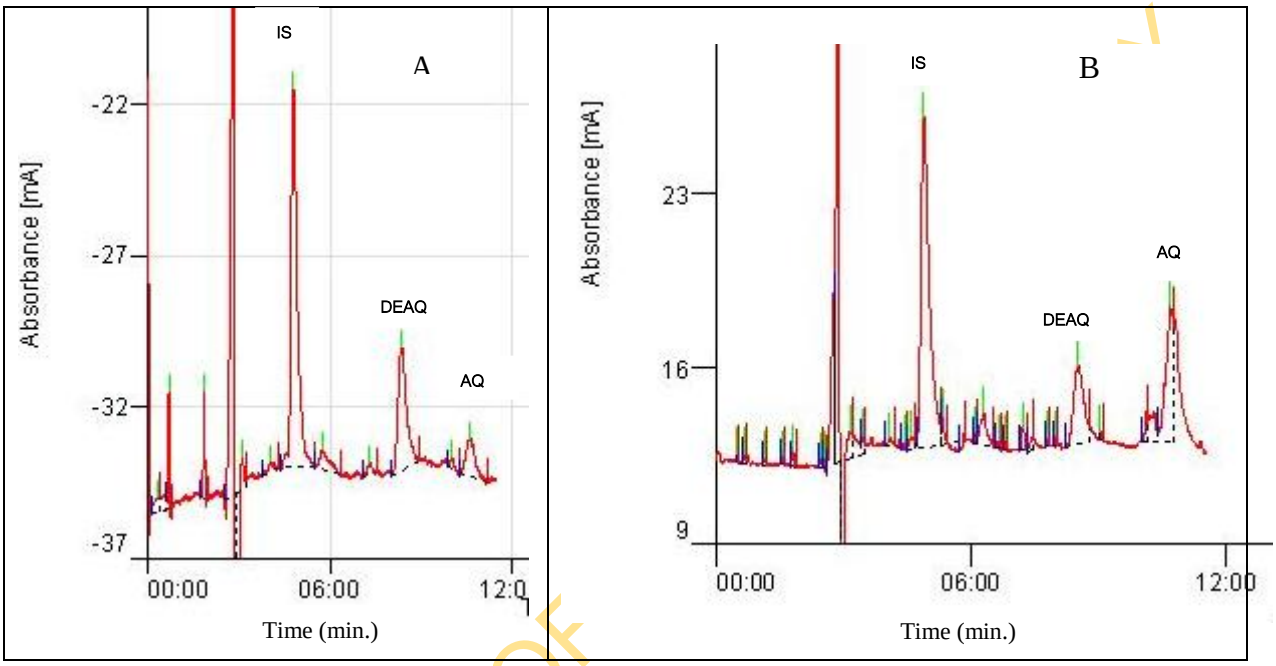

Figure 4.18: Chromatograms showing amodiaquine (AQ) and desethylamodiaquine (DEAQ) in plasma (A) and saliva (B) of a patient at 4 h after the first dose of oral artesunate-amodiaquine. Quinidine is the internal standard (IS)

4.25: Patients enrolled in the study

The characteristics of seven children who were randomly selected from the group of children treated with artesunate-amodiaquine combination (30mg/kg of amodiaquine base over 3 days that is 10mg/kg daily) are shown in Table 4.29. The seven children enrolled in the study aged between 6-13 years. The mean age was 10.8 ± 2.8 years and mean weight was 26.2 ± 5.9kg. The geometric mean parasite density was 43797/µl of blood. The pH of saliva generally ranged between 6.8 and 7.8 in all children.

Table 4.29: Baseline clinical and demographic characteristics of seven children with acute uncomplicated falciparum malaria enrolled in the study.

Parameters	Mean±SD
Age (y)	10.8±2.8
Range	7 – 13
Weight (kg)	26.2±5.9
Range	16 – 33
Gender (F:M)	4:3
Axillary Temperature (^{O}C)	38.2±0.65
Range	36.9 – 38.6
% PCV	32.6±4
Range	29 – 39
Geometric Mean of parasite density /µl of blood	43797
Range	19,009 – 150,000

SD= standard deviation, PVC= pack cell volume,

4.26: Pharmacokinetic parameters of Desethyl amodiaquine in children with uncomplicated falciparum malaria after administration of standard doses of artesunate-amodiaquine

Two of the seven children who were enrolled had AQ and DEAQ in their blood pre-dosing and were excluded from the analysis. Therefore data from 5 children out of the 7 who were enrolled were used for the pharmacokinetic analysis. Amodiaquine was rapidly converted to desethylamodiaquine, which appeared in the plasma and saliva within 40 minutes in all subjects. All children had detectable levels of amodiaquine in plasma and saliva up to 40 h. The plasma and saliva concentration-time curve for desethylamodiaquine for the five children are shown in Figure 4.19. The concentration on day 7 was 210.3ng/ml in plasma and 131.8ng/ml in saliva (Table 4.30). The decline phases of the desethylamodiaquine in saliva concentration-time curves were approximately parallel to that in plasma (Figure 4.19). The saliva concentration was approximately a quarter and two-fifth that in plasma.

The pharmacokinetic parameters derived from the oral plasma concentration-time curve are summarized in Table 4.31. The saliva pharmacokinetic parameters are summarized in Table 4.32. The plasma half-life (t1/2) was 156.7±20.6h (s.e.m). The elimination half-life from saliva was also similar at 139.1±8.3h. There was no significant difference between plasma and saliva half-life (P = 0.16) (Table 4.33).

The mean AUC were 96003.6 ± 12344.6 ng/ml.h (s.e.m) for plasma and 74004.2 ± 9514.3 ng/ml.h for the saliva and were similar (P = 0.196) (Table 4.33). Comparison of other pharmacokinetic parameters in the 5 patients gave a mean volume of distribution (Vd) of 74.1 ± 16.2 L/kg (s.e.m) for plasma and 91.5±21.8 L/kg for DAQ in saliva. The plasma oral clearance calculated from the expression CLp=fD/AUC,

was found to be 325.4±48.8ml/h/kg (s.e.m). This, too, was not significantly different from the clearance of 443.7±85.1ml/h/kg obtained from saliva data.

Table 4.30: Saliva-plasma ratio of desethyl amodiaquine concentrations in five children with falciparum malaria treated with standard oral doses of artesunate-amodiaquine.

Desethyl amodiaquine concentration (ng/ml)				
Time (days)	**Plasma (n=5)**	**Saliva (n=5)**	**Saliva/plasma ratio**	**P value**
0	0	0	ND	ND
7	210.3±31.59*	131.8±19.5	0.626	0.068
	121.9 – 314.9**	75.5 – 176.1		
14	193.9±47.1	141.0±24.0	0.727	0.358
	98.7 – 293.3	92.5 – 183.4		
28	111.3±48.1	41.4±24.0	0.119	0.237
	63.1 – 159.4	9.0 – 88.5		
35	27.6±5.4	6.4±3.1	0.233	0.064
	19.2 – 37.7	3.3 – 9.6		

*Mean±sem, ** Range

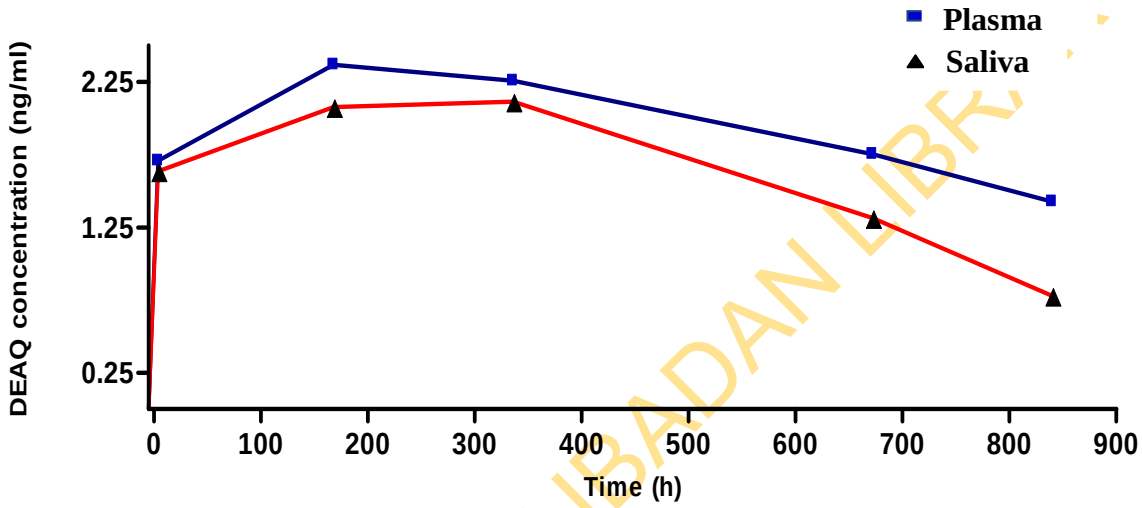

Figure 4.19: Plasma and Saliva log concentration-time profile of desethylamodiaquine (DEAQ) in children infected with *P. falciparum* malaria who were treated with oral doses of artesunate-amodiaquine.

Table 4.31: Pharmacokinetic disposition of desethyl-amodiaquine in plasma after standard oral doses of artesunate-amodiaquine (30mg/kg amodiaquine) in five children with uncomplicated malaria.

Subject (n=5)	Weight (Kg)	$t_{1/2}$ (h)	Vd (Lkg^{-1})	Cl ($mlh^{-1}kg^{-1}$)	AUC_{0-35d} ($ng.ml^{-1}h$)	Extrapolated AUC (%)
1	27	132.9	87.2	454.8	71370.0	2.6
2	29	233.3	131.2	389.7	70859.4	11.0
3	20	128.8	43.5	234.0	124593.5	2.7
4	29	121.5	62.4	355.9	87015.4	0.2
5	33	167.2	46.5	192.8	126179.8	10.7
Mean ±sem	27.6±2.1	156.7±20.6	74.1±16.2	325.4±48.8	96003.6±12344.6	5.4±2.2

s.e.m= Standard error of mean

Table 4.32: Pharmacokinetic disposition of desethylamodiaquine in saliva after standard oral doses of artesunate-amodiaquine (30mg/kg amodiaquine) in five children with uncomplicated malaria.

Subject (n=5)	Weight (Kg)	$t_{1/2}$ (h)	Vd (Lkg^{-1})	Cl ($mlh^{-1}Kg^{-1}$)	AUC_{0-35d} ($ngml^{-1}h$)	Extrapolated AUC (%)
1	27	133.6	66.7	346.0	94390.0	2.0
2	29	135.8	119.2	608.1	50376.9	1.2
3	20	114.5	52.3	316.7	90743.9	4.1
4	29	165.7	164.2	686.6	42804.1	5.2
5	33	146.1	55.0	260.9	85843.5	17.8
Mean±sem	27.6±2.1	139.1±8.3	91.4±21.8	443.7±85.1	72831.7±10864.5	6.1±3.0

s.e.m= Standard error of mean

Table 4.33: Comparison of pharmacokinetic disposition of desethyl amodiaquine in plasma and saliva after standard oral doses of artesunate-amodiaquine (30mg/kg amodiaquine) in children with uncomplicated malaria (n=5).

Parameters	Plasma n=5	Saliva n=5	P value
$t_{1/2}$ (h)*	156.7 ± 20.6	139.1 ±8.3	0.453
95% CI	99.3 – 214.2	115.8 – 162.4	
Range**	121.5 – 233.3	114.5 – 165.7	
AUC_{0-35d} ($ngml^{-1}.h$)	96003.6±12344.661	72831.7±10864.54	0.196
95% CI	729.3 – 130277.9	2666.8–102996.6	
Range	87015.4 – 126179.9	42804.1 – 94390.0	
CL ($mLh^{-1}Kg^{-1}$)	325.4±48.8	443.7±85.1	0.302
95% CI	189.4-461.1	207.8-680.2	
Range	192.8 – 454.8	260.9 – 680.1	
Vd (LKg^{-1})	74.1 ± 16.2	91.5 ± 21.8	0.542
95% CI	29.1 – 119.2	30.8 – 152.1	
Range	43.5 – 131.2	52.3 – 164.2	

*Mean±sem, ** Range

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Appendices

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Table 4.a: Standard curve table for extrapolation of unknown concentration of chloroquine in whole blood on filter paper.

Concentration (ng/mL)	CQ peak Height (mV)	Papaverine (PPV) peak Height (mV)	Ratio of CQ/PPV peak Height
0	0	6.1	0
100	2.8	6.4	0.4375
200	6.5	6.7	0.9701
400	12.4	6.8	1.8235
800	21.2	6.2	3.4193
1000	26.1	5.8	4.5000
2000	48.3	5.3	9.1320

$r^2 = 0.99158$

Table 4.b: Standard curve table for extrapolation of unknown concentration of sulphadoxine in whole blood on filter paper

Concentration (µg/mL)	Sulphadoxine Peak Height (mV)	Sulisoxazole (IS)Peak Height (mV)	Ratio of SDX/SXZ Peak Heights
0	0	110.9	0
10	45.9	107.0	0.4289
20	123.6	171.7	0.7198
40	146.8	145.3	1.0103
50	220.0	147.0	1.4959
60	219.9	106.9	2.0579

r^2= 0.99158

Table 4.c: Standard curve table for extrapolation of unknown concentration of desethyl amodiaquine in plasma.

Concentration (ng/ml)	IS (QND) peak height	DAQ peak height	Peak heights DEAQ/QND ratio
0	31.6	-	0.00
100	36.7	3.2	0.08
200	38.7	4.9	0.12
400	41.7	13.0	0.31
600	36.0	18.3	0.50
800	37.1	22.5	0.60

r^2= 0.9907

Table 4.d: Standard curve table for extrapolation of unknown concentration of desethyl amodiaquine in saliva.

Concentration (ng/ml)	IS (QND) peak height	DEAQ peak height	Peak height DEAQ/QND ratio
0	30.9	-	0.00
100	32.4	13.6	0.41
200	24.4	14.6	0.59
400	28.8	29.4	1.02
600	27.9	42.8	1.53
800	28.7	60.0	2.09

r^2= 0.9838