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Pharmacokinetic Characteristics of Two Paediatric Formulations of Artesunate-Mefloquine in African Children with Acute Uncomplicated *Plasmodium falciparum* Malaria

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2 Introduction

More than 3 billion people worldwide live in areas at risk of malaria. An estimated 350-500 million clinical malaria cases occur annually [1]. In Africa the majority of infections are caused by *Plasmodium falciparum*, the most virulent of the four human malaria parasites. Moreover Africa hosts the most effective malaria vector, the mosquito *Anopheles gambiae*. As a result, more than one million people in Sub-Saharan Africa die from malaria each year. Most of them are young children. Every fifth death of an African child is caused by *Plasmodium falciparum* malaria, making the disease the fourth greatest cause of death in under five year olds in Africa [2]. For the last years, this disease burden has increased, a circumstance that is partly owed to the spread of drug-resistant parasites. Commonly used antimalarials, such as most aminoquinolines, have become increasingly ineffective, leading to an urgent need for new treatment options. Following current recommendations, these new regimens should be combination therapies.

2.1 Antimalarial Combination Therapy

Antimalarial combination therapy is defined as “simultaneous use of two or more blood schizontocidal drugs with independent modes of action” [3].

There are three main reasons for the concept of combining antimalarials: first to increase efficacy, second to shorten duration of treatment, and third to delay development of resistance to antimalarial drugs [4].

The enhancement of therapeutic efficacy has been a major issue since drug resistant strains of *Plasmodium falciparum* have rendered former antimalarial monotherapeutic regimens ineffective. Simultaneous administration of two independent drugs has proven to overcome this decreased efficacy of monotherapy by acting on different biochemical targets.

The potential of drug combinations to shorten duration of treatment has been shown in a number of clinical trials [5]. A further advantage thereby is that patients' compliance is improved with shorter treatment courses, which is again related to effectiveness and development of drug resistance.
The major point regarding resistance is, however, that drug combinations can reduce the emergence of resistant parasites. The so-called mutual simultaneous protection is – at least in theory – based on a simple calculation: resistance develops, when spontaneously occurring parasite mutants with diminished drug susceptibility are selected and transmitted. The probability that a mutant will arise, which is resistant to two different drugs at the same time, is by far lower than the emergence of a mutant, which is resistant to one drug alone [6].

2.1.1 Artemisinin Containing Combinations

Among the variety of possible antimalarial combinations artemisinin-based combination therapies (ACT) have particularly been advocated during the last years. Fast reduction of the parasite biomass, quick resolution of clinical symptoms and reduction of gametocyte carriage are some of the striking advantages of these artemisinin-containing regimens [7].

Artesunate-mefloquine is one of the most thoroughly examined antimalarial combinations and much of the early field experience of artemisinin-based combinations was gained with this treatment regimen, especially in South East Asia. When in 1994, after 10 years of use, the efficacy of high-dose (25mg/kg) mefloquine monotherapy in the border regions of Thailand had decreased to less than 70%, introduction of 4mg/kg per day artesunate treatment for three days combined with 25mg/kg mefloquine resulted in nearly 100% efficacy [8]. From that time cure rates with this regimen stayed above 90% for almost a decade. As shown by Brockman *et al*. [9] there has even been a significant improvement of mefloquine *in vitro* sensitivity in isolates from this area.
Overall artesunate-mefloquine has proven to be a highly effective drug combination for the treatment of *Plasmodium falciparum* malaria in South East

Asia [10-14]. Its potency might even have contributed to a decline in the incidence of *Plasmodium falciparum* malaria in certain areas [8]. Yet, recent data from *in vivo* sensitivity monitoring in Thailand showed reduced efficacy of only 78.6% adequate parasitological and clinical response after 28 days in one province [15]. Mey Bouth Denis and his colleagues [16] reported on similar findings of decreased efficacy after artesunate-mefloquine treatment in Cambodia, both raising questions about the future of the therapy in this area.
The combination has also been investigated in South America proving good tolerability and high efficacy in Peru and Bolivia [17, 18]. In Africa the combination was found to be highly efficacious, yet data from clinical trials are limited, especially for children [3, 19, 20]. To what extent experience from South East Asia can be transferred to hyperendemic areas in sub-saharan Africa remains an open question [4, 21]. It is therefore important to obtain reliable data for artesunate-mefloquine treatment of acute uncomplicated *Plasmodium falciparum* malaria in African children who represent the main target group worldwide.

Artemether-lumefantrine (benflumentol), another currently available artemisinin combination, was the first ACT to be registered as a fixed-dose treatment (i.e. two drugs in one tablet) according to international guidelines. Studies from Asia suggested high efficacy and good safety and tolerability, although the combination tends to be less efficacious than artesunate-mefloquine [22-24]. Moreover it must be given as a complex six-dose regimen, which curtails its usefulness in the field.

Treatment of uncomplicated malaria with artesunate and amodiaquine is recommended by WHO for areas, where efficacy of amodiaquine monotherapy is not less than 80%. Out of 37 African countries, which adopted ACTs as first line therapy, 15 have chosen artesunate-amodiaquine [3, 25]. Yet a randomised multicentre trial in African children found only limited efficacy and there is an ongoing debate on the regimen’s safety [26].

Artesunate combined with sulfadoxine-pyrimethamine is another inexpensive artemisinin-based combination. It was adopted as first line treatment by Mozambique and Sudan. Results from clinical trials in African children, especially from East Africa, are rather disappointing [7] in contrast to data from Mali where the regimen was recently found to be as efficacious as artemether-lumefantrine [27]. This discrepancy can most likely be explained by differences in the rate and extent of resistance against sulfadoxine-pyrimethamine in the respective study regions.

Dihydroartemisinin-piperaquine is a new combination regimen under evaluation which is increasingly deployed in South East Asia. Randomised controlled clinical trials indicated excellent efficacy as well as a good safety and tolerability profile [28-32]. Karema and colleagues [33] recently confirmed these findings for children from Rwanda. In one study from Thailand, polymerase chain reaction corrected cure rates on day 63 after dihydroartemisinin-piperaquine treatment were superior to cure rates after artesunate-mefloquine treatment [28]. The combination is available as fixed-dose formulation for a simple, once daily regimen. The combination of dihydroartemisinin and piperaquine is a very promising therapy and might be a good new alternative to current artemisinin-containing treatments, especially as it is far less expensive than artesunate-mefloquine or artemether-lumefantrine.

Overall, high efficacy and good tolerability of artemisinin-based combination therapies make an important contribution to the control of *Plasmodium falciparum* malaria worldwide. The question, however, which ACT or whether at all ACT is the best option for the official first-line recommendation of an individual country – especially in Africa – has not been adequately studied in most cases.

General disadvantages of artemisinin-based combinations are high prices and limited availability of artemisinin-derivatives. Moreover all ACT-regimens combine artemisinin-drugs, which have relatively short plasma elimination half-

lives, with drugs that have comparably long elimination half-lives. All “partner” drugs stay in the blood for several days, mefloquine and piperaquine even for several weeks. Especially in areas with high intensity of transmission, widespread use of such combinations would result in exposure of parasites to low doses of those drugs in case of reinfection after treatment. This, however, leads to an increased risk for the emergence and spread of drug resistance.

Table 1 Elimination half-lives of drugs used as combination partner of artemisinin- derivatives

drug	elimination half-life
mefloquine [34]	20 days
piperaquine [35]	21 days
amodiaquine [36]	10.1 days
lumefantrine [37]	3.2 days
sulfadoxine [38]	4.4 days
pyrimethamine [38]	2.5 days
artesunate/ dihydroartemisinin [39]	approx. 1 hour

data are depicted as approximate mean values
data from studies in children were used as far as available

The “pharmacokinetic mismatch”[4] of all current ACT suggests on the one hand that new partner drugs with equally short $t_{1/2}$ such as clindamycin be considered as combination partners for artemisinins [40]. On the other hand, non-ACT regimens should be reassessed for their use in Africa.

2.1.2 Non-Artemisinin-Based Combinations

Quinine + tetracycline is a non-artemisinin-based combination of two drugs with quite similar elimination half-lives (see table below). It has been used for the treatment of *Plasmodium falciparum* malaria over a long time, yet the seven-day treatment course with several drug-intakes every day causes problems with compliance. Moreover the combination cannot be used in children and pregnant women. More options for two drugs with more or less similar plasma half-lives would be quinine + clindamycin [40], atovaquon and proguanil [41], or the combination of fosmidomycin and clindamycin [4, 42].

Other non-ACT regimens, which are still being widely used in Africa, are quinine + sulfadoxine-pyrimethamine and sulfadoxine-pyrimethamine + amodiaquine. Efficacy of both treatments depends on the level of resistance to the single components and therefore differs considerably, depending on the region where the treatment is deployed [43, 44]. In a very recent study from Burkina Faso, where sulfadoxine-pyrimethamine combined with amodiaquine was equally high efficacious as artemether-lumefantrine, Zongo *et al.* [45] showed that non-ACTs are still highly effective in some parts of Africa. Given the fact that they are less expensive and more available, their use as alternatives to artemisinin-based combination treatments can still be prudential.

Table 2 Elimination half-lives of drugs in non-artemisinin combinations

drug	elimination half-life
quinine [46]	8-10 hours
tetracycline [47]	8-10 hours
atovaquon [41]	1-2 days
proguanil [41]	12-15 hours
clindamycin [48]	2-4 hours
fosmidomycin [49]	3-4 hours

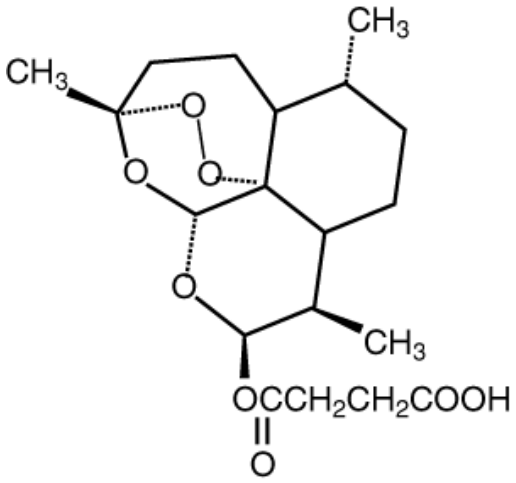
2.2 The Combination of Artesunate and Mefloquine

Following current WHO guidelines, the treatment of *Plasmodium falciparum* malaria with the combination of artesunate-mefloquine is recommended as a three day treatment course of 12mg/kg artesunate (4 mg/kg per day) and a total of 25 mg/kg mefloquine [3].

2.2.1 Artesunate

The endoperoxide-containing artemisinin and its derivatives artesunate, artemether, arteether and dihydroartemisinin are among the most important drugs in antimalarial chemotherapy.

In 1972 Chinese scientists isolated artemisinin from *Artemisia annua*, the sweet wormwood or quinghao, which had been used by Chinese herbal medicine practitioners for more than 2000 years.



Artesunate

In the face of rapid emergence of *Plasmodium falciparum* strains resistant to commonly used antimalarials, artemisinin derivatives soon became widely used in South East Asia, showing remarkably high activity such as rapid resolution of fever and parasitaemia, even in multidrug resistant *Plasmodium falciparum* malaria. Nevertheless artesunate monotherapy is associated with high levels of parasitological failure rates and five to seven day treatment regimens are needed to prevent recrudescent parasitaemia [5, 14, 50].

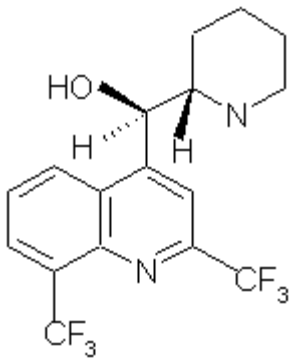
Artesunate is a water-soluble hemisuccinate ester of the original sesquiterpene lactone artemisinin. It can be administered orally, as suppository, intravenously with 5% sodium bicarbonate solution or intramuscularly as artesunic acid. Bioavailability of artesunate after oral administration is reported to range between 60 and 90% [51, 52]. As for all artemisinin derivatives,

dihydroartemisinin (DHA) appears to be the principal active metabolite of artesunate *in vivo*. Biotransformation of artesunate to dihydroartemisinin is very rapid, yet the degree at which artesunate is transformed varies considerably. Hydroxylation of artesunate to dihydroartemisinin is pH dependent and seems to occur already in the gastrointestinal tract, which means that the drug is partly absorbed in form of its metabolite. In the blood, hydrolysis of artesunate is catalysed by esterases [53]. Dihydroartemisinin binds moderately to human plasma proteins (43%), predominantly to albumin [54]. It is quickly eliminated from plasma with an elimination half-life time of approximately one hour. Dihydroartemisinin undergoes glucuronidation in liver microsomes, primarily mediated by uridine-glucuronyltransferases 1A9 and 2B7 and is mainly excreted via the urine as alpha-dihydroartemisinin-ß-glucuronide [55].

Artemisinin derivatives are most effective against late ring to early trophozoite stages [56]. Considerable evidence exists that their mode of action involves iron catalysed decomposition of the labile endoperoxide bridge into free radicals [54].
Recent work by Eckstein Ludwig and colleagues [57] suggests that the SERCA-type PfATPase6, a sarco-/endoplasmatic reticulum Ca^{++}-ATPase, be the specific target for artemisinins. This was lately supported by Jambou *et al*. [58], who described *in vitro* resistance of *Plasmodium falciparum* field isolates from Senegal and French Guiana to artemether connected with point mutations of the SERCA PfATPase6 gene.

2.2.2 Mefloquine

The synthetic 4-quinolinmethanol mefloquine was synthesized by the Walter Reed Army Institute in the 1960ies, when resistance to chloroquine caused major problems in the Vietnam war. From that time it has proven to be an effective antimalarial for both prophylaxis and treatment.



Mefloquine

Increasing resistance especially in the border regions of Thailand and worries about toxicity such as adverse reactions of the central nervous system have meanwhile been limiting its use.

The lipophilic drug, consistent of a quinoline nucleus with an amino-alcohol side chain, is a weak base with a pka1 value below 2 and a pka2 value of 8.6. It acts against the intra-erythrocytic asexual stages of all species of human malaria parasites. Although mefloquine has been used in the field for more than 30 years, its exact mode of action is still not entirely clear. Like chloroquine and the other quinoline antimalarials it is thought to block the detoxification of IronIIIprotoporphyrin IX (FP), which is released during digestion of the hosts haemoglobin in the *Plasmodium* lysosomal food vacuole. Whether this is mainly achieved through binding to haematin [59], through “capping” of haematin polymers by FP-mefloquine complexes [60], through interaction with the peroxidative- or glutathione-dependent degradation of haem [61, 62] or through a different mechanism awaits final elucidation.

Mefloquine is distributed extensively in tissues and eliminated slowly with a terminal elimination half-life around 20 days. Plasma protein binding exceeds 98% [34]. Cytochrom P450 3A seems to be involved in transformation of mefloquine to its 2 major metabolites carboxy-mefloquine and hydroxyl-mefloquine [63]. As there is no parenteral formulation for mefloquine, its absolute oral bioavailability in humans cannot be determined.

2.2.3 Artesunate-Mefloquine Dose- and Regimen Finding

Several studies have been conducted in order to determine the appropriate dosage regimen for the combination treatment of artesunate and mefloquine. It was shown that a three day course of 4 mg/kg artesunate and a total mefloquine dose of 25 mg/kg is superior to shorter courses of artesunate or lower doses of mefloquine or to either drug alone [10, 14, 64]. It has been discussed controversially, whether mefloquine should be given simultaneously from the first day (8,5mg/kg for three days) or sequentially (15 mg/kg on the second and 10 mg/kg on the third day of treatment). In a randomized controlled trial conducted by François Nosten and colleagues [10] in Thailand, vomiting could be significantly reduced by administration of mefloquine on days 2 and 3. Contrary to that, a double blind study in Thailand found no difference in the occurrence of adverse events such as vomiting between two groups with sequential and simultaneous administration [13]. Interestingly, it was shown in another randomized, double-blind multi-centre trial conducted in Africa that the occurrence of vomiting was significantly lower in patients with simultaneous dosing compared to the group with sequential administration of mefloquine on day 2 and 3 [19]. Latest results from population pharmacokinetic assessment indicate, that oral bioavailability of mefloquine is best, when the 25 mg/kg dose is split into three doses of 8 mg/kg each [65].

Concerning pharmacokinetic interactions between the two drugs, early work by Karbwang *et al*. [66] and Price *et al.* [67] suggested that artesunate could influence absorption, distribution and metabolism of mefloquine. Recent studies, however, revealed evidence for the fact that there is no significant interference between the two compounds. This means that simultaneous administration of both drugs does not influence pharmacokinetic properties of either component [68-70].

2.3 Formulation of Combination Treatment Regimens

Besides effectiveness, safety, tolerability and accessibility of antimalarials, adherence to treatment has been characterized as major determinant for successful treatment of malaria, especially when dealing with rural populations in developing countries [71-74]. In general, compliance with sequential combination regimens of antimalarial drugs tends to be problematic with patients being loath to take antimalarials after clinical improvement. This is a particular danger for artemisinin-containing combinations, as they resolve clinical symptoms such as fever quicker than all other antimalarials [5]. This relief of symptoms might easily be interpreted as cure, making patients reluctant to continue treatments as prescribed, which quickly leads to recrudescent parasitaemia.

In a Study from Myanmar, Shwe and colleagues [75] showed that provision of blister packing can be a very effective way to improve compliance and consequently to increase effectiveness of combination treatments. In this study pre-packed single blisters with daily doses of both components, artesunate and mefloquine, significantly increased adherence and completion of the three day treatment course. Yeboah-Antwi *et al*. [76] reported on similar findings from a study conducted in West Africa.

Development of fixed-dose co-formulations is another step forward in the effort to provide suitable formulations for successful unsupervised treatment of malaria in the field. Co-formulation of both drugs together in one tablet eliminates the possibility of patients taking only one component of the combination. This could be of particular importance for the treatment of African children with combinations that contain quinoline compounds, such as mefloquine: due to their unpleasant taste, they are often rejected by young patients, which might make mothers tend to omit administration of the bitter mefloquine pill, once the artemisinin has abated symptoms of their sick child [77].

There are currently only two artemisinin-based fixed-dose combinations available: artemether-lumefantrine and dihydroartemisinin-piperaquine, and

only the former has international registration. Two more fixed-dose co-formulations are under development, one for the combination of artesunate-amodiaquine and one for artesunate-mefloquine [65, 78].

2.4 Study Objectives

Artemisinin-based combination therapy is now the recommended treatment for uncomplicated *Plasmodium falciparum* malaria. Appropriate drug formulation is an essential factor for the success of the strategy of artemisinin-based combination therapy and particularly new fixed-dose combinations are urgently needed.

The aim of our study was to evaluate pharmacokinetic properties of two paediatric oral formulations of artesunate and mefloquine: Standard tablets of 100 mg artesunate and 250 mg mefloquine in a co-blister for African children of 20 to 40 kg body weight, and a new paediatric fixed-dose formulation of 50 mg artesunate and 125 mg mefloquine for children between 10 and 20 kg body weight.

Both treatments are simple, once-daily regimens for a three day treatment course, formulated specially for the use in paediatric patients.

The new fixed combination is an oral preparation of taste-masked granules, developed for young children who have difficulties to swallow tablets. It addresses the need for practical, user-friendly fixed-dose ACT drug regimens for paediatric malaria outpatients.

Facilitated drug administration should help to ameliorate patients' compliance, thereby increase effectiveness of treatment and reduce the risk of emergence and spread of resistance.

So far there have been no reports on pharmacokinetics of artemisinin-based antimalarials in African children. Data of our study therefore aimed to enhance the knowledge about these drugs in an important target population.

3 Methods

The pharmacokinetic evaluation of artesunate-mefloquine combination therapy was part of an open-label, stratified clinical trial on the efficacy, safety and pharmacokinetic characteristics of two paediatric formulations of artesunate-mefloquine in African children with acute uncomplicated *Plasmodium falciparum* malaria. The study took place at two centres in Gabon from October 2005 to February 2006.

A total of 71 patients were enrolled in this clinical trial: 40 children were included at the Centre Hospitalier de Libreville and 31 at the Medical Research Unit of the Albert Schweitzer Hospital in Lambaréné. Pharmacokinetic analysis was done in 24 patients. All of them were enrolled at the Medical Research Unit in Lambaréné.

3.1 Study Site

Lambaréné is a town of about 20.000 inhabitants located amid the dense Central African rainforest of Gabon at 0° 30´south. The area is characterized by a perennial hyperendemic *Plasmodium falciparum* transmission [79] with an entomological inoculation rate of approximately 50 infective bites per person per year [80].

Plasmodium falciparum isolates are highly resistant to chloroquine, as shown in a number of *in vitro* and *in vivo* studies [81-85].

Quinine is the drug of choice for most hospitalized patients and many outpatients. Despite broad administration for a long time, susceptibility to the drug has remained high. [82, 83, 85]

Mefloquine is a less commonly used antimalarial in this region. In the early 1990ties 2 *in vitro* studies showed its good activity against *Plasmodium*

falciparum in the study area with full inhibition of schizont maturation of all isolates below the threshold level of resistance (6.4µmol/l) [82, 83].
During a clinical trial in 1994 mefloquine treatment at 15mg/kg exhibited 100% efficacy in children from Lambaréné with uncomplicated malaria [86]. Another *in vitro* study again found no mefloquine resistant isolates [87]. In 2002 Ramharter and colleagues [85] carried out an *in vitro* susceptibility test with 41 isolates from the study area and reported on comparably decreased but still sufficient susceptibility of *Plasmodium falciparum* to mefloquine.

Data for activity of artemisinins is limited for the study area. In 1994 Philipps *et al.* [83] reported on good susceptibility of *Plasmodium falciparum* isolates to artesunate *in vitro.* In a clinical trial, addition of 3 day oral artesunate treatment (4mg/kg) to amodiaquine (10mg/kg for three days) could improve efficacy at day 28 compared to amodiaquine monotherapy from 71% to 85% [26]. A short course treatment of 4 mg/kg artesunate monotherapy once daily for three days exhibited Polymerase Chain Reaction corrected cure rates of 92% by day 14 and 72% by day 28 [50].
Results from an *in vitr*o susceptibility study in 2003 suggest 1.6 ng/ml and 9.7 ng/ml as 50% and 90% effective concentrations for dihydroartemisinin respectively [88] (molecular weight of 284.4 used for transformation of results).

3.2 Trial population

The study comprised of a total of 71 African children, able to take oral medication and suffering from uncomplicated *Plasmodium falciparum* malaria. 41 patients with a body weight between 10 and 20 kg were assigned to treatment group A and 30 patients with 20 to 40 kg body weight to treatment group B. Pharmacokinetic characteristics of dihydroartemisinin and mefloquine were investigated in the first 12 patients in each group who accepted and were suitable to participate in the pharmacokinetic part of the study.

Patients were deemed eligible for the study if they met the following Inclusion criteria:

- Male or female with a body weight between 10 and 40 kg
- Patients suffering from acute uncomplicated *Plasmodium falciparum* malaria
- Malaria diagnosis confirmed by a positive blood smear with asexual forms of *Plasmodium falciparum* (i.e., identification of asexual parasite count ≥1,000 to 250,000 per mm^3 blood)
- Ear temperature ≥ 37.5°C or a history of fever within the last 48 hours
- Haemoglobin ≥ 7g/100ml
- Written informed consent from parents or guardian.

The following patients were ineligible for participation in this clinical trial (exclusion criteria) :

- Patients with signs and symptoms of severe/complicated malaria requiring parenteral treatment defined according to WHO Recommendations [89]
- Patients with known hypersensitivity or allergy to artemisinin derivatives or mefloquine or mefloquine chemically related compounds (for example quinine and quinidine)
- Patients who had received quinine or any artemisinin derivatives within 12 hours prior to study start
- Patients who had received any other adequate antimalarial drug therapy including antibiotics which might be active against malaria infection within 1 week prior to study start
- Patients who had received investigational (unlicensed) drugs as well as mefloquine within 30 days prior to study start
- Patients with known history of psychiatric disorders
- Patients with known history of cardiac diseases and arrhythmia
- Patients with known sickle cell disease

- Patients with clinical signs of or laboratory evidence for any other severe hepatic, renal, pulmonary, cardiac, metabolic, psychiatric, cancer or haematological diseases
- Females during pregnancy or lactation

3.3 Investigational Drugs

The investigational therapy consisted of daily doses of artesunate ranging from 2.5 to 5 mg/kg/day (mean total dose 12 mg/kg after three days as recommended by WHO[3]) and daily doses of mefloquine ranging from 6.25 to 12.5 mg/kg/day (mean total dose 25 mg/kg) given simultaneously once daily for 3 days.

Patients in group A (10-20 kg) received once a day the new oral fixed-dose combination of 50 mg artesunate and 125 mg mefloquine. The new paediatric formulation is a taste-masked preparation of granules of artesunate and mefloquine put together in one single stick-pack, which contains the daily dose for a 3 day treatment course for children up to 20 kg bodyweight. The granules are mango flavoured and can be administered directly on to the child's tongue. Once in the mouth, the granules dissolve into a gel like substance, making it easy for the child to swallow the drug.

Patients in group B (>20-40 kg) were treated with the standard co-blister tablets of 100mg artesunate and 250mg mefloquine respectively. The tablets were taken with a reasonable amount of water and without chewing. The daily dose was administered by one of the investigators and administration time and acceptance were documented. All study drugs were provided by Mepha Ltd. (Mepha Pharmaceutical Research, Aesch/Basel, Switzerland)

The dosing scheme is presented in the table below:

Table 3 Dosing scheme

	Treatment Group A ≥10 – 20 kg new paediatric formulation	Treatment Group B >20 – 40 kg standard co-blister tablets
Day 1	1 Stickpack containing 50 mg artesunate and 125 mg mefloquine	1 tablet artesunate 100 mg and 1 tablet mefloquine 250 mg
Day 2	1 Stickpack containing 50 mg artesunate and 125 mg mefloquine	1 tablet artesunate 100 mg and 1 tablet mefloquine 250 mg
Day 3	1 Stickpack containing 50 mg artesunate and 125 mg mefloquine	1 tablet artesunate 100 mg and 1 tablet mefloquine 250 mg

In case of vomiting within one hour after drug intake, the full dose was replaced. Patients who vomited after drug administration were excluded from pharmacokinetic assessment.

3.4 Investigational Plan

For all patients who took part in the clinical trial, the study design comprised baseline assessment, treatment period and follow-up.

The Baseline period included informed consent processus, parasitological examination (i.e. thick blood film), physical examination, laboratory safety tests, ECG and pregnancy tests.

The treatment period started with administration of the first study drug on day 1, when all baseline assessments had been done and all inclusion criteria were fulfilled. The second dose was given 24 hours later on day 2 and the third and last drug administration 48 hours after the first dose on day three. During the treatment period all patients were hospitalized in the study centre for regular

performance of thick blood films, physical examination, laboratory safety tests, ECGs, surveillance of vital signs and observation of adverse events.
After day three patients were followed-up for 28 days. During weekly visits on day 7, 14, 21 and 28 vital signs, body temperature and occurrence of adverse events were assessed and thick blood films for parasitological examination were taken.

3.5 Pharmacokinetic Analysis

In order to determine the plasma concentration versus time profile of dihydroartemisinin and mefloquine, blood samples were taken at 9 time points from the 24 patients assigned for pharmacokinetic evaluation. For dihydroartemisinin a blood sampling scheme was chosen to allow demonstration of a complete pharmacokinetic profile of the drug. Therefore blood was collected at 30 minutes, 60 minutes, 90 minutes, 2 hours, 4 hours and 6 hours after first drug administration on day 1.
Given the long half-life of mefloquine, for ethical reasons a sparse sampling schedule was defined allowing to demonstrate the average plasma levels reached at specific time points but not a formal pharmacokinetic analysis. Blood samples for mefloquine were taken 6 hours after first treatment on day one, 54 hours after first treatment (i.e. 6 hours after treatment on day three) and finally on day 28, approximately 650 hours after application of the first study treatment. In addition 2 predose samples were taken just before administration of the first study drug.
The following scheme presents study design and time points of blood sampling for dihydroartemisinin and mefloquine analysis:

Scheme 1 Study design

	Baseline	*Treatment period*												*Follow-up period*			
Days	0	1								2		3		7	14	21	28
Hours	-1	0	0.5	1	1.5	2	4	6	12	24	36	48	54				648
DHA sampling	**x**		**x**	**x**	**x**	**x**	**x**	**x**									
Mefloquine sampling	**x**							**x**					**x**				**x**
Study medication		x								x		x					
Blood film	x					x	x	x	x	x	x	x		x	x	x	x
Vital signs	x								x	x	x	x		x	x	x	x
ECG	x												x				x
Laboratory tests	x												x				x
Physical exam	x												x	x			x

For each blood sample 4 ml of whole blood were put into Lithium-heparinized polypropylene tubes (Sarstedt, Nümbrecht, Germany), gently inverted several times, and centrifuged immediately. After centrifugation for 10 min at approximately 2000 x gravity, the supernatant plasma (approximately 2x1 ml) was pipetted off, transferred in two polypropylene tubes, labelled and deep frozen at ≤-70°C as soon as possible. The tubes were kept frozen at ≤ -70°C pending analysis.
For blood samples on day 1 an indwelling cannula (BD, Franklin Lakes/NJ, USA) was inserted in a forearm vein. Samples on day 3 and day 28 were taken by direct stick.

3.6 Drug Assays

Analysis of plasma samples was done by High Performance Liquid Chromatography (HPLC), a form of column chromatography, used to separate components of a mixture based on a variety of chemical interactions between the analytes and the chromatography column.
Chemical compounds mixed in the plasma sample are injected into the column and come out of the instrument at specific time points, depending on the different retention times of the molecules. This allows to detect and to quantify the separated compounds such as drugs and their metabolites by analysing the peaks that the molecules provoke at those characteristic time points.
In our study we used reverse-phase HPLC (RP-HPLC), where the so called stationary phase in the analytical column is nonpolar and therefore hydrophobic analytes tend to be retained on the column.

Concentrations of dihydroartemisinin were determined by HPLC with online Solid Phase Extraction (OSP) and tandem mass spectroscopy (LC/MS/MS).
The following equipment and reagents were used:

Pump 2149 for sample injection (LKB-Pharmacia, Piscataway/NJ, USA), Auto sampler 231XL (Gilson-Abimed, Middleton/WI, USA), Dilutor 401c and 402 (Gilson-Abimed) and Column Oven BFO-04 Svn 1 (Jasco, Easton/MD, USA). Before quantification, DHA was extracted from plasma by solid-phase extraction with Inertsil OSD 10µ extraction column (Knauer 4 x 5mm, MZ Analysentechnik, Mainz, Germany).
The chromatographic analysis was done using Inertsil OSD2 C-18 analytical column (125 x 3mm, MZ Analysentechnik) with acetonitrile (Roth 35978, Karlsruhe, Germany) as mobile phase, delivered at a flow rate of 0.35 ml/min at 35˚C for 5 min (400µl) with isocratic elution.
Mass spectroscopy was carried out on a API 2000 Detector (Applied Biosystems /MDS Sciex, Foster City/CA, USA) in the multi-reaction-monitoring (MRM) mode. The extracted ion for DHA was m/z 302. Artemisinin (400µg/ml) was used as Internal Standard.

For analytical detection of mefloquine HPLC with UV detection was performed, using the following equipment:
Pump 880 PU (Jasco), Auto sampler 231 (Gilson-Abimed), Dilutor 401 (Gilson-Abimed) and Column Oven 665A-52 (Merck/Hitachi, Tokyo, Japan). The solid phase extraction was carried out by using Perisorb RP-2 extraction column (Merck, Darmstadt, Germany). Samples were incubated with acetonitrile (1.00030.2500 Merck) as mobile phase and diluted with distilled water before being delivered at a flow rate of 1 ml/min at 50˚C in isocratic mode (350µl). Analysis was done using Inertsil OSD2 C-18 HPLC column, 125 x 4 mm (MZ Analysentechnik) and mefloquine was detected at 222nm wave length with detector UV-2075 (Jasco).

3.7 Analytical and Statistical Plan

Dihydroartemisinin and mefloquine concentrations in plasma were analysed at the time points specified for each individual of the two treatment groups. Pharmacokinetic analysis was performed using standard non-compartmental methods (Kinetika 2000/3.0, Inna Phase Corp, Philadelphia/PA, USA) . The following parameters were calculated for dihydroartemisinin: the observed maximum plasma concentration C_{max}, the time to peak plasma concentration T_{max}, the area under the plasma concentration versus time curve to the last sample with quantifiable drug concentration AUC_{0-t}, the area under the concentration-time curve extrapolated to infinity $AUC_{0-\infty}$, elimination constant λz, which was estimated for all datasets where at least three concentration-time points in the terminal elimination slope (ß phase) were available, and terminal elimination half-life $t_{1/2}$, computed as $\ln 2/\lambda z$. The elimination constant λz was calculated by log-linear regression employing the method of least-squares.

For mefloquine pharmacokinetic analysis was essentially restricted to descriptive statistics of plasma levels at the respective sampling time points, as the number of samples planned per protocol did not allow further analysis.

Descriptive statistics including minimum, median, maximum, geometric mean, arithmetic mean, 90% confidence limits for the arithmetic mean and 10% and 90% quantiles were calculated according to distribution of data. Comparison of groups, and linear regression analysis of mefloquine drug concentrations were performed (JMP 5.0, SAS Institute Inc, NC, USA).

3.8 Ethics and Good Clinical Practice

Before study implementation the protocol and informed consent were reviewed and approved by the Ethics Committee of the International Foundation of the Albert Schweitzer Hospital. The study was carried out in compliance to the protocol, in accordance with the Declaration of Helsinki and in adherence to Good Clinical Practice guidelines [90].

4 Results

4.1 Study flow

24 symptomatic children were recruited for the pharmacokinetic assessment and received a complete course of treatment. One patient in group A was withdrawn from the study and treated with rescue medication due to repeated vomiting on the first day of treatment. He was excluded from pharmacokinetic analysis and replaced by an additional patient. All patients responded rapidly to treatment. 24 hours after the first dose, 15 patients (66.5%) had completely cleared parasitaemia. 48 hours after initiation of treatment thick blood smears of all patients were negative and none of the children developed recrudescent parasitaemia until day 28.

Clinical baseline characteristics of patients are shown in Table 4. Biochemical and haematological parameters were within the expected range for African children suffering from uncomplicated *Plasmodium falciparum* malaria.
Following inclusion criteria, patients in group A were of younger age and lower body weight than in group B.

Table 4 Characteristics of PK-patients upon admission

	Group A	Group B
Admission characteristics		
No of participants	12	12
No of female participants	7	4
Age (years)	5.0 (2.3-6.0)	10.5 (6.3-12.0)
Weight (kg)	15.5 (11.4-18.7)	27.0 (21.6-36.7)
Height (cm)	104 (92-113)	130 (120-154)
Body surface (m^2)	0.66 (0.55-0.77)	0.99 (0.85-1.25)
Body Mass Index (kg/m^2)	14.5 (11.6-16.2)	15.2 (14.1-17.8)
Initial Parasitaemia (per µl)	25,385 (1,289-173,325)	33,553 (2,781-84,510)
Temperature (°C)	37.6 (36.8-39.7)	37.3 (37.0-38.4)
Heart Rate	130 (83-148)	91 (61-119)
Systolic Pressure	93.5 (76.4-106.9)	102 (88-116)
Diastolic Pressure	65.0 (40.4-74.7)	68 (55-76)
Haematology		
Haematocrit (%)	27.3 (22.3-33.4)	29.7 (27.4-35.7)
Haemoglobin (g/dl)	9.3 (7.7-11.1)	10.2 (9.4-12.2)
Reticulocyte count (%)	0.96 (0.47-1.63)	0.33 (0.23-0.72)
Platelets	278 (117-512)	209 (67-511)
WBC	5.1 (3.1-9.9)	7.5 (4.4-12.6)
Biochemistry		
Bilirubin (µmol/l)	9.0 (3.9-31.3)	12.0 (4.4-25.9)
Creatinine (µmol/l)	34.9 (18.8-48.6)	41.3 (23.3-71.2)
Alkaline Phosphatase (U/l)	199 (158-379)	189 (95-271)
Glucose (mmol/l)	3.8 (1.7-5.6)	4.0 (3.2-4.7)
ALAT (U/l)	17.5 (9.6-43.3)	22.0 (12.2-39.3)
ASAT (U/l)	28.5 (19.3-104.1)	29.5 (23.3-99.1)

All data are depicted as median (10% and 90% quantiles).

Within the two groups, the bodyweights of the study population were well distributed: for the fixed-dose paediatric formulation (suitable for children from 10-20 kg) study patients had a median bodyweight of 15.5 kg, ranging from 11-19 kg. In group B (standard co-blister for children >20-40 kg) the patients' median body weight was 27.0 kg, ranging from 21 to 37 kilos.
This resulted in median daily doses of 3.2 mg/kg and 3.7 mg/kg artesunate in group A and B respectively. The median total dose of mefloquine was 24.2 mg/kg in group A and 28.1 mg/kg in group B. Table 5 gives a summary of daily and total dosages in both treatment groups.

Table 5 Dosages of artesunate and mefloquine

	group A		group B	
	median	range	median	range
Daily artesunate (mg/kg)	3.2	2.6-4.5	3.7	2.7-4.8
Total artesunate (mg/kg)	9.7	7.9-13.4	11.2	8.1-14.3
Daily mefloquine (mg/kg)	8.1	6.6-11.2	9.4	6.8-11.9
Total mefloquine (mg/kg)	24.2	19.7-33.5	28.1	20.3-35.7

4.2 Pharmacokinetics of Artesunate/Dihydroartemisinin

All of the planned 168 blood samples for the determination of dihydroartemisinin in plasma were taken, yet two tubes had to be discarded due to labelling problems.

Table 6 shows pharmacokinetic parameters of the children treated with either the fixed-dose formulation or the co-blister tablets:

Table 6 Dihydroartemisinin pharmacokinetic parameters

	Group A (fixed-dose formulation)				Group B (co-blister tablets)			
	Mean [90%CI]	Median	min	max	Mean [90%CI]	Median	min	max
T_{max} (h)	--	1.5	1.0	6.1	--	1.5	0.5	4.1
C_{max}[1] (ng/ml)	812 [673-1689]	861	130	3390	878 [736-1425]	930	286	2190
AUC_{0-t}[1] (h*ng/ml)	1763 [1539-3305]	2050	130	6641	2151 [1810-3045]	2470	965	4781
$AUC_{0-\infty}$[1,2] (h*ng/ml)	2867 [2186-4129]	3024	1399	6679	2679 [2086-4200]	2815	1049	7791
$t_{1/2}$[2] (h)	--	0.9	0.6	2.2	--	1.0	0.6	10.1
λz [2]	0.8 [0.60-0.90]	0.8	0.3	1.1	0.67 [0.52-0.82]	0.7	0.1	0.7

--Mean and confidence intervals not computed due to not-normal distribution of data
[1] Means and 90% confidence intervals are depicted as antilog of arithmetic mean of transformed data using the natural logarithm
[2] $t_{1/2}$ $AUC_{0-\infty}$ and λz were computed for all datasets where at least three points in the terminal slope (β-phase) were available (n=9 in group A, n=11 in group B)

Overall the parameters for dihydroartemisinin appear quite similar between both formulations. In either group artesunate was rapidly absorbed and transformed into dihydroartemisinin (median T_{max} 1.5h in both groups). Median peak plasma concentrations for dihydroartemisinin were 861 ng/ml and 930 ng/ml in group A and B, respectively.

3 patients in group B showed peak dihydroartemisinin plasma levels as early as 30 minutes after drug intake (1550, 933, 1719 ng/ml). The latest time point for C_{max} in this group was at 4h in patient B4 (286ng/ml).
In contrast to that, 3 patients in group A reached C_{max} not until 6 hours after drug administration, thus at the latest sampling point for dihydroartemisinin. Observed maximum plasma concentrations in these patients were comparatively low (158, 130, 420 ng/ml). As a consequence there was a remarkable inter-individual variation of C_{max} (range 130-3390 ng/ml, 26 fold variation in group A, 286-2190 ng/ml, 7.5 fold variation in group B). According to

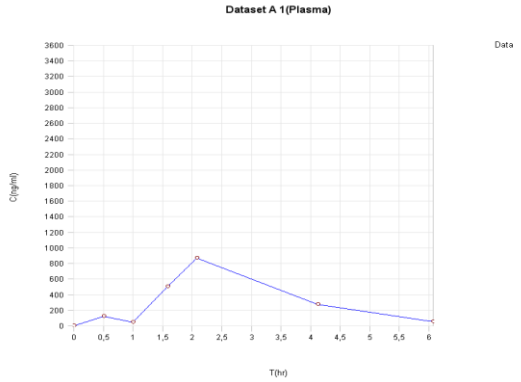
that AUC_{0-t} values ranged from 130 to 6641 h*ng/ml for patients in group A and from 965 to 4781 h*ng/ml in group B. Median AUC_{0-t} levels were 1763 and 2151 h*ng/ml in group A and B, respectively.

For the 4 patients showing delayed absorption (3 in group A, 1 in group B) elimination constant λz, $t_{1/2}$, and $AUC_{0-\infty}$ could not be computed. Median $AUC_{0-\infty}$ values of the remaining participants in treatment group A (3024 ng/ml) and B (2815 ng/ml) were comparable. The terminal half-life of dihydroartemisinin was 0.9 and 1.0 h for the two drug formulations, respectively.

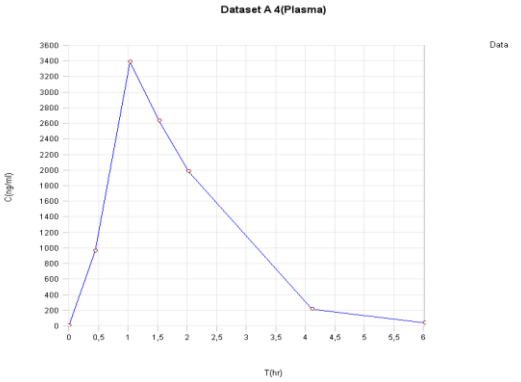
Although the study was not designed and powered for bioequivalence testing, comparative analysis of pharmacokinetics has been performed in a post hoc analysis. The A : B ratio of geometric mean values of peak dihydroartemisinin concentrations was 0.93 (90% CI 0.52-1.66), the ratio of AUC_{0-t} 0.81 (90% CI 0.48-1.39), indicating slightly higher bioavailability of formulation B.
Pharmacokinetic parameters were more influenced by gender than by treatment groups. Median C_{max} for male patients in group A were 737 ng/ml (10% and 90% quantiles 130-1700) in comparison to 922 ng/ml (10% and 90% quantiles 420-3390) in females (p=0.12). In group B median peak plasma concentrations for female patients were more than 3 times higher than for males (1730 ng/ml for females, 10% and 90% quantiles 927-2190, and 543 ng/ml for males, 10% and 90% quantiles 286-1710, p=0.04).
There was a significant positive correlation between the quantity of daily artesunate doses and $AUC_{0-\infty}$ in group B (r=0.78, p=0.005) but not in group A (r=0.12). For other clinical parameters such as parasitaemia, temperature, biochemical and haematological determinants, no significant influence on pharmacokinetic characteristics was found in either treatment group.

The following figures (figures 1-7) show dihydroartemisinin plasma concentration versus time curves for the individual patients and mean plasma concentration/time profiles for the 2 treatment groups:

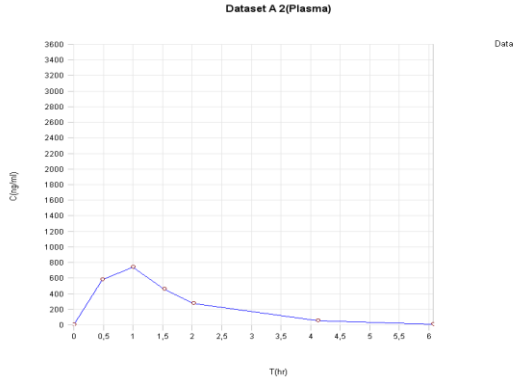
Figure 1 Dihydroartemisinin plasma concentrations of patients A1-A6



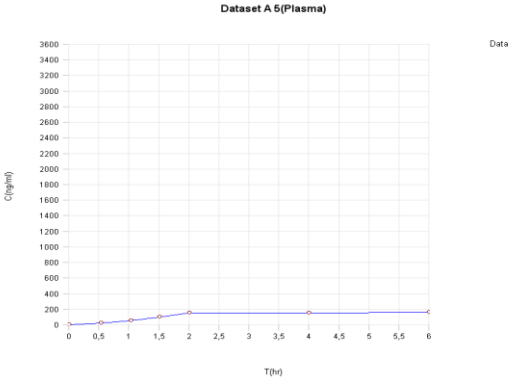
gender	m	C_{max} (ng/ml)	868
age (ys)	5	AUC_{0-t} (h*ng/ml)	2047
weight (kg)	15	$AUC_{0-\infty}$ (h*ng/ml)	2116
height (cm)	101	$t_{1/2}$ (h)	0.97



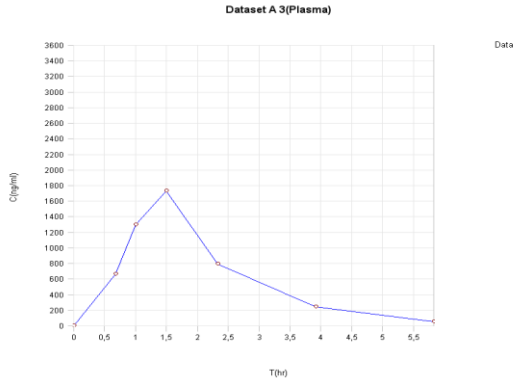
gender	f	C_{max} (ng/ml)	3390
age (ys)	4	AUC_{0-t} (h*ng/ml)	6640
weight (kg)	14.5	$AUC_{0-\infty}$ (h*ng/ml)	6679
height (cm)	98	$t_{1/2}$ (h)	0.71



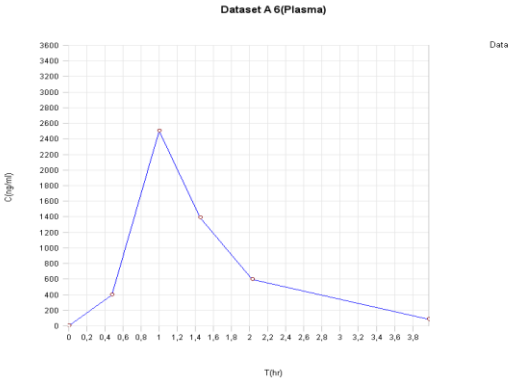
gender	m	C_{max} (ng/ml)	737
age (ys)	3	AUC_{0-t} (h*ng/ml)	1386
weight (kg)	16	$AUC_{0-\infty}$ (h*ng/ml)	1399
height (cm)	99	$t_{1/2}$ (h)	0.86



gender	m	C_{max} (ng/ml)	158
age (ys)	5	AUC_{0-t} (h*ng/ml)	733
weight (kg)	16	$AUC_{0-\infty}$ (h*ng/ml)	--
height (cm)	104	$t_{1/2}$ (h)	--

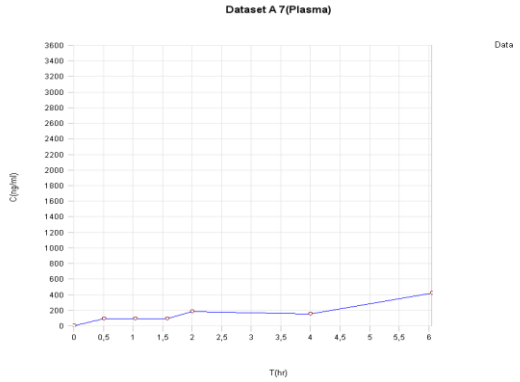


gender	f	C_{max} (ng/ml)	1730
age (ys)	6	AUC_{0-t} (h*ng/ml)	3457
weight (kg)	16	$AUC_{0-\infty}$ (h*ng/ml)	3526
height (cm)	106	$t_{1/2}$ (h)	0.90

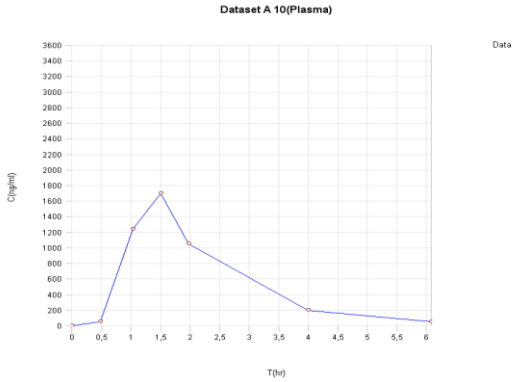


gender	f	C_{max} (ng/ml)	2500
age (ys)	6	AUC_{0-t} (h*ng/ml)	2953
weight (kg)	19	$AUC_{0-\infty}$ (h*ng/ml)	3024
height (cm)	113	$t_{1/2}$ (h)	0.61

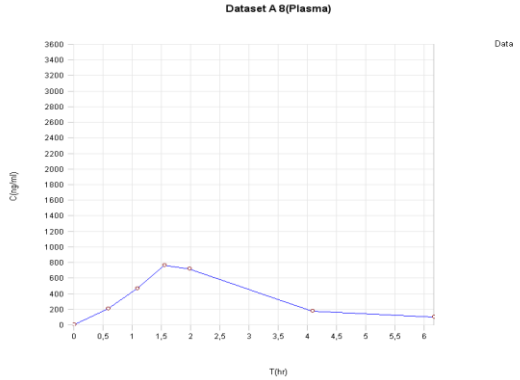
Figure 2 Dihydroartemisinin plasma concentrations of patients A7-A12



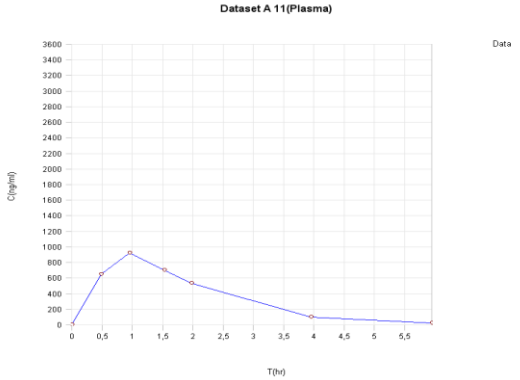
gender	f	C_{max} (ng/ml)	420
age (ys)	5	AUC_{0-t} (h*ng/ml)	1109
weight (kg)	15	$AUC_{0-\infty}$ (h*ng/ml)	--
height (cm)	105	$t_{1/2}$ (h)	--



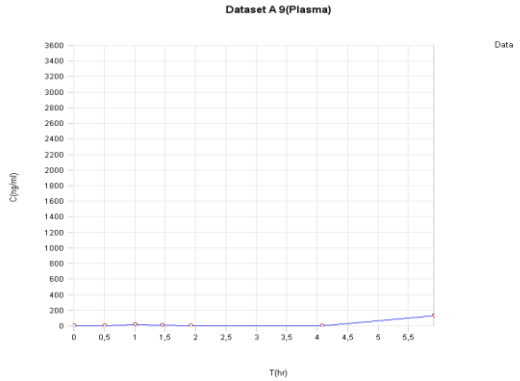
gender	m	C_{max} (ng/ml)	1700
age (ys)	6	AUC_{0-t} (h*ng/ml)	3244
weight (kg)	18	$AUC_{0-\infty}$ (h*ng/ml)	3312
height (cm)	113	$t_{1/2}$ (h)	0.91



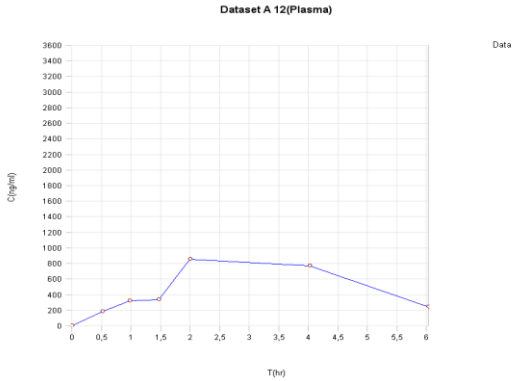
gender	f	C_{max} (ng/ml)	764
age (ys)	5	AUC_{0-t} (h*ng/ml)	2053
weight (kg)	12	$AUC_{0-\infty}$ (h*ng/ml)	2257
height (cm)	104	$t_{1/2}$ (h)	1.45



gender	f	C_{max} (ng/ml)	922
age (ys)	6	AUC_{0-t} (h*ng/ml)	2010
weight (kg)	14	$AUC_{0-\infty}$ (h*ng/ml)	2039
height (cm)	105	$t_{1/2}$ (h)	0.87

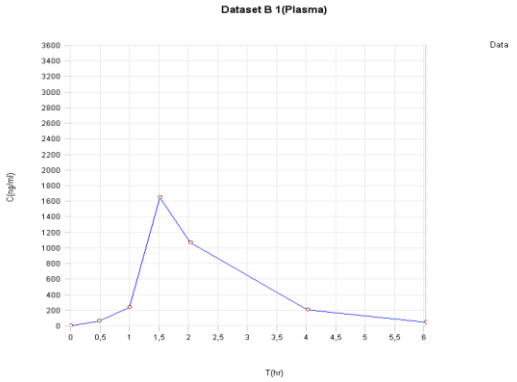


gender	m	C_{max} (ng/ml)	130
age (ys)	3	AUC_{0-t} (h*ng/ml)	130
weight (kg)	16	$AUC_{0-\infty}$ (h*ng/ml)	--
height (cm)	100	$t_{1/2}$ (h)	--

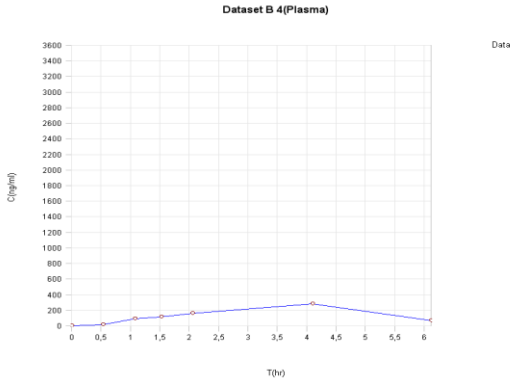


gender	f	C_{max} (ng/ml)	854
age (ys)	2	AUC_{0-t} (h*ng/ml)	3300
weight (kg)	11.2	$AUC_{0-\infty}$ (h*ng/ml)	4068
height (cm)	90	$t_{1/2}$ (h)	2.21

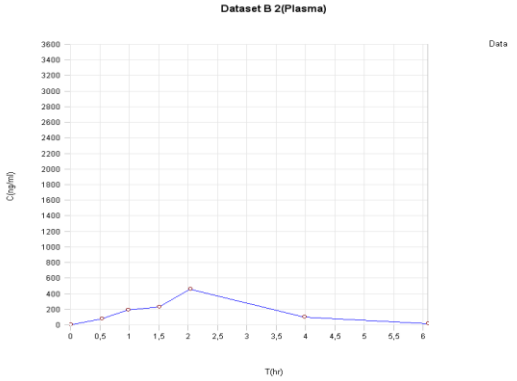
Figure 3 Dihydroartemisinin plasma concentrations of patients B1-B6



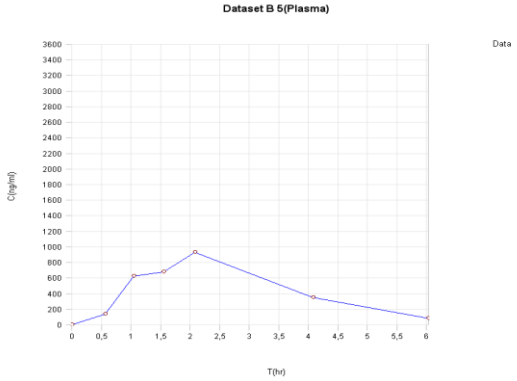
gender	f	C_{max} (ng/ml)	1650
age (ys)	10	AUC_{0-t} (h*ng/ml)	2800
weight (kg)	24	$AUC_{0-\infty}$ (h*ng/ml)	2858
height (cm)	120	$t_{1/2}$ (h)	0.87



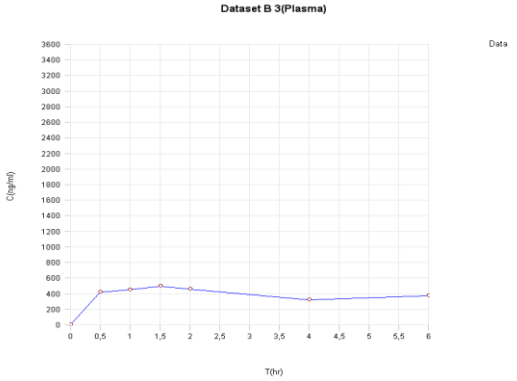
gender	m	C_{max} (ng/ml)	286
age (ys)	11	AUC_{0-t} (h*ng/ml)	965
weight (kg)	23	$AUC_{0-\infty}$ (h*ng/ml)	--
height (cm)	129	$t_{1/2}$ (h)	--



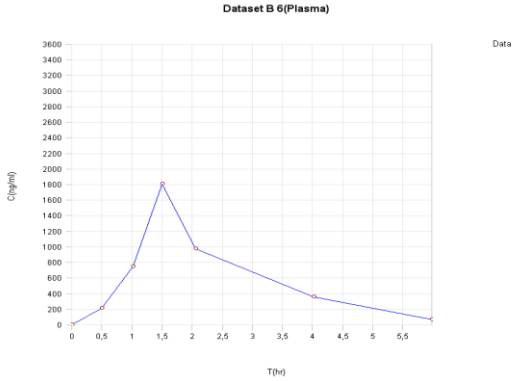
gender	m	C_{max} (ng/ml)	459
age (ys)	11	AUC_{0-t} (h*ng/ml)	1031
weight (kg)	35	$AUC_{0-\infty}$ (h*ng/ml)	1049
height (cm)	152	$t_{1/2}$ (h)	0.82



gender	f	C_{max} (ng/ml)	927
age (ys)	12	AUC_{0-t} (h*ng/ml)	2676
weight (kg)	31	$AUC_{0-\infty}$ (h*ng/ml)	2823
height (cm)	130	$t_{1/2}$ (h)	1.14

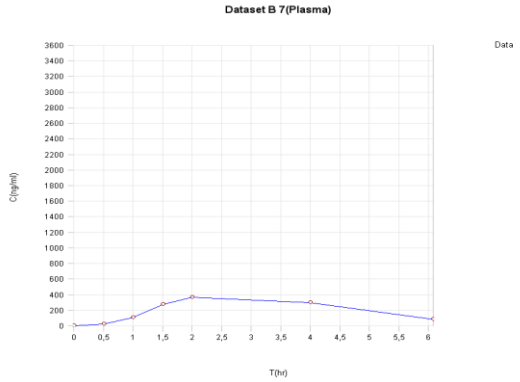


gender	m	C_{max} (ng/ml)	496
age (ys)	6	AUC_{0-t} (h*ng/ml)	2278
weight (kg)	23	$AUC_{0-\infty}$ (h*ng/ml)	7791
height (cm)	123	$t_{1/2}$ (h)	10.14

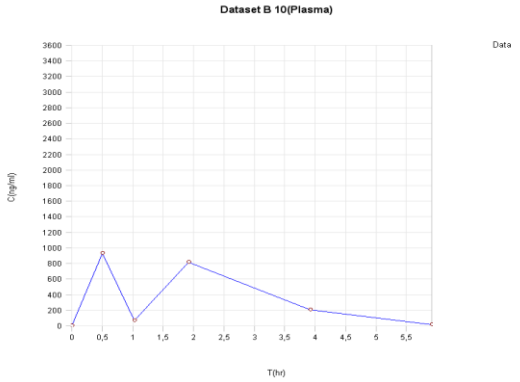


gender	f	C_{max} (ng/ml)	1810
age (ys)	7	AUC_{0-t} (h*ng/ml)	3420
weight (kg)	21	$AUC_{0-\infty}$ (h*ng/ml)	3522
height (cm)	120	$t_{1/2}$ (h)	1.00

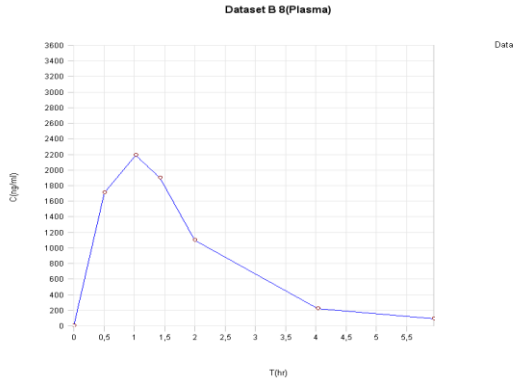
Figure 4 Dihydroartemisinin plasma concentrations of patients B7-B12



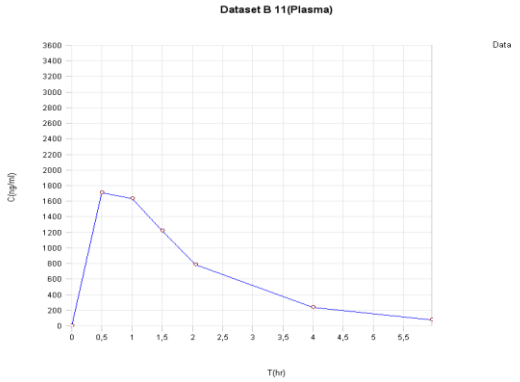
gender	m	C_{max} (ng/ml)	365
age (ys)	11	AUC_{0-t} (h*ng/ml)	1343
weight (kg)	30	$AUC_{0-\infty}$ (h*ng/ml)	1568
height (cm)	141	$t_{1/2}$ (h)	1.89



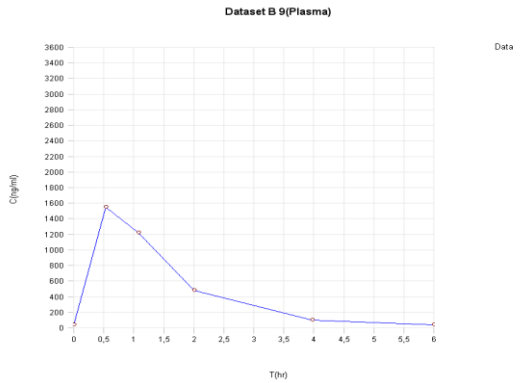
gender	m	C_{max} (ng/ml)	933
age (ys)	9	AUC_{0-t} (h*ng/ml)	2136
weight (kg)	30	$AUC_{0-\infty}$ (h*ng/ml)	2156
height (cm)	140	$t_{1/2}$ (h)	0.73



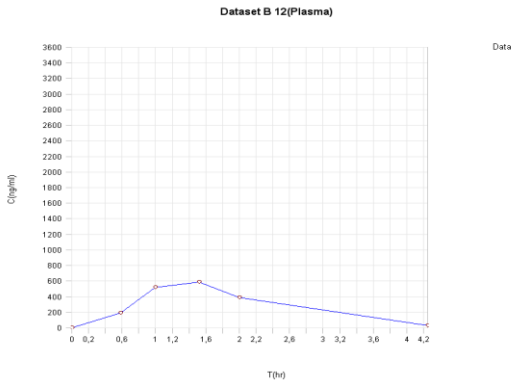
gender	f	C_{max} (ng/ml)	2190
age (ys)	10	AUC_{0-t} (h*ng/ml)	4781
weight (kg)	24	$AUC_{0-\infty}$ (h*ng/ml)	4916
height (cm)	125	$t_{1/2}$ (h)	1.02



gender	m	C_{max} (ng/ml)	1710
age (ys)	8	AUC_{0-t} (h*ng/ml)	3829
weight (kg)	24	$AUC_{0-\infty}$ (h*ng/ml)	3954
height (cm)	128	$t_{1/2}$ (h)	1.16



gender	m	C_{max} (ng/ml)	1550
age (ys)	11	AUC_{0-t} (h*ng/ml)	2662
weight (kg)	37	$AUC_{0-\infty}$ (h*ng/ml)	2710
height (cm)	152	$t_{1/2}$ (h)	0.96



gender	m	C_{max} (ng/ml)	590
age (ys)	12	AUC_{0-t} (h*ng/ml)	1205
weight (kg)	36	$AUC_{0-\infty}$ (h*ng/ml)	1236
height (cm)	155	$t_{1/2}$ (h)	0.65

Figure 5 Dihydroartemisinin plasma concentrations for patients of group A (fixed-dose formulation)

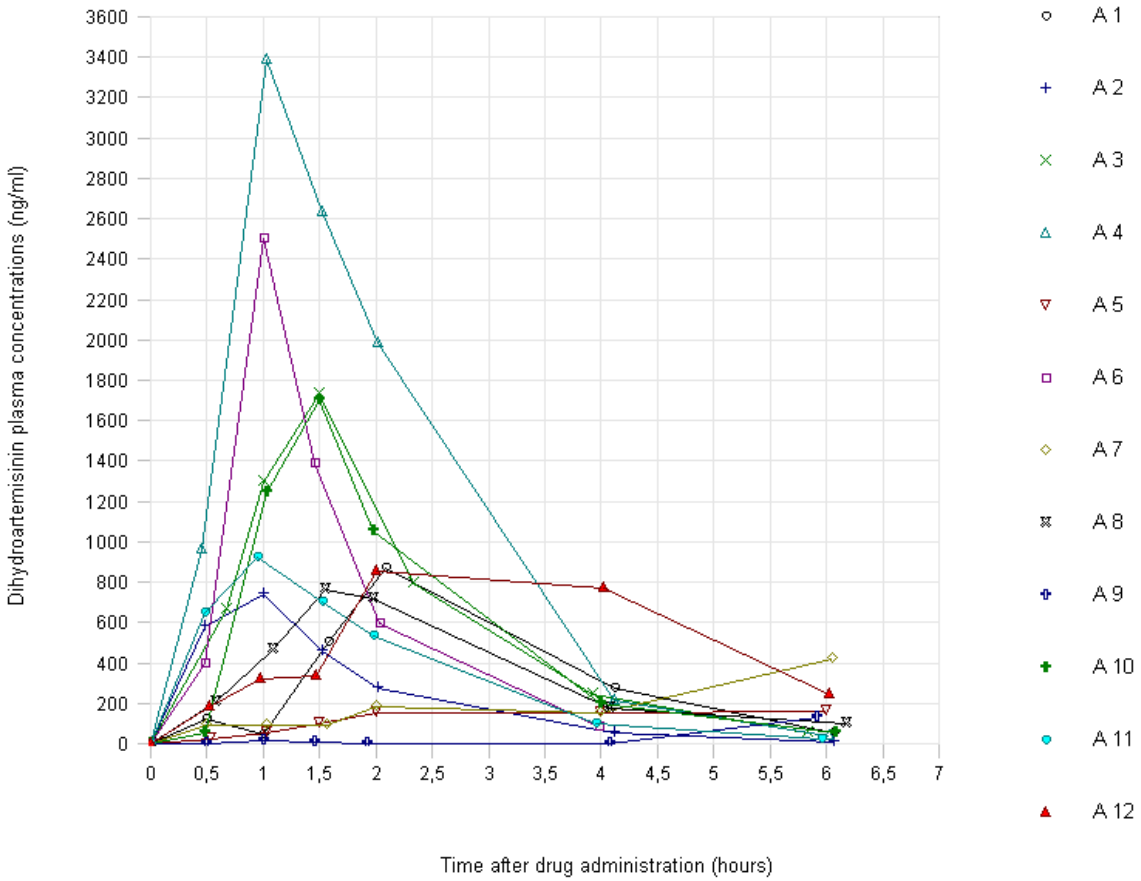


Figure 6 Dihydroartemisinin plasma concentrations for patients of group B (co-blister tablets)

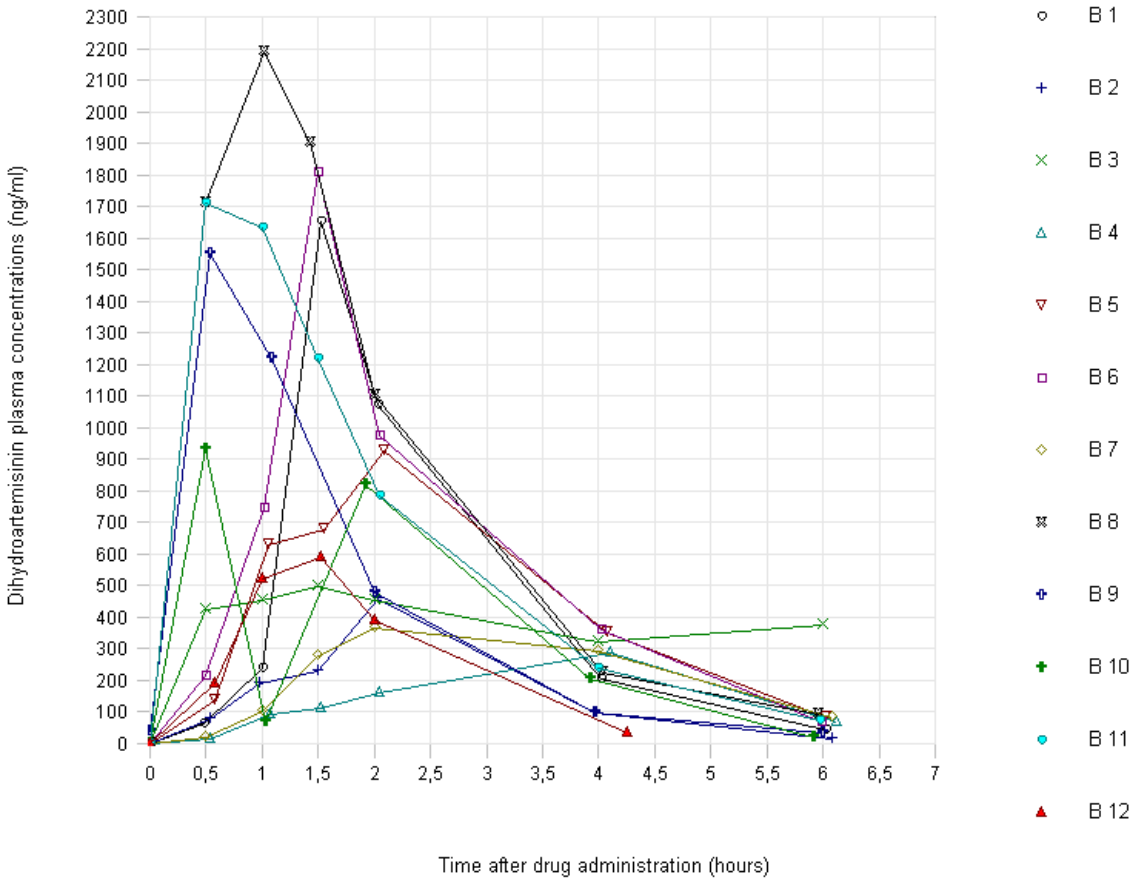
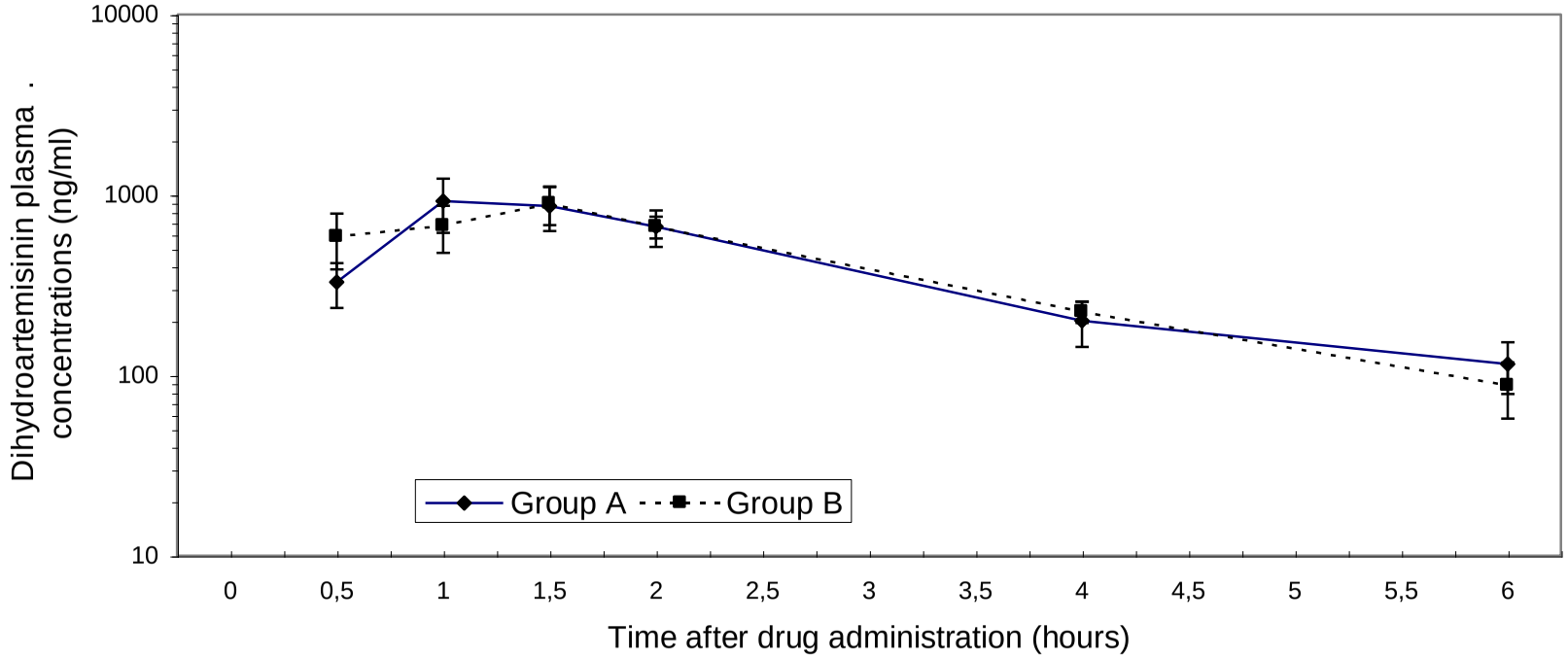


Figure 7 Mean plasma-concentration versus time profile of dihydroartemisinin for treatment groups A (fixed-dose formulation) and B (co-blister tablets).



Time points for plasma sampling are depicted on x-axis.

Log transformed mean plasma concentrations and standard error of the mean are depicted on y-axis

The influence of dihydroartemisinin plasma levels on parasite- and fever reduction was assessed in post-hoc analysis. Therefore individual relative parasite reduction within the first 6 and 12 hours after treatment on day one as well as fever clearance time and time until total parasite clearance were correlated with dihydroartemisinin peak plasma levels and the area under the concentration-time curve.

In general there was no strong correlation between dihydroartemisinin plasma levels and efficacy parameters. In group A, however, higher peak plasma levels were associated with a higher rate of parasite reduction within the first 6 hours after drug intake (r=0.51, p=0.08). For patients in group B, however, the correlation between parasite reduction and C_{max} was negative (-0.54, p=0.07). In group B, patients with higher peak plasma levels and higher AUC_{0-t} values tended to have shorter fever clearance times (r=-0.78, p=0.12 for C_{max}; r=-0.89 p=0.04 for AUC_{0-t}), whereas in group A no such correlation could be found. Table 7 shows correlations between dihydroartemisinin pharmacokinetic parameters and parasite- and fever reduction.

Table 7 Spearman's rank correlation coefficients ρ between dha-phramacokinetics and parameters of parasite- and fever reduction

	Group A (fixed-dose formulation)				Group B (co-blister tablets)				
	PRR[1] 6h(%)	PRR[2] 12h(%)	PCT[3] (hours)	FCT[4] (hours)	PRR[1] 6h(%)	PRR[2] 12h(%)	PCT[3] (hours)	FCT[4] (hours)	
C_{max}	**0.52 p=0.08**	0.23 p=0.48	0.24 p=0.46	-0.16 p=0.70	-0.54 p=0.07	-0.48 p=0.11	0.28 p=0.38	**-0.78 p=0.12**	C_{max}
AUC_{0-t}	0.29 p=0.37	-0.04 p=0.89	0.40 p=0.19	0.15 p=0.72	-0.43 p=0.16	-0.35 p=0.27	0.23 p=0.47	**-0.89 p=0.04**	AUC_{0-t}

[1] Parasite Reduction Rate within the first 6 hours after treatment on day one, computed as 1- (Parasitaemia at 6h / Parasitaemia Predose)
[2] Parasite Reduction Rate within the first 12 hours after treatment on day one, computed as 1- (Parasitaemia at 12h / Parasitaemia Predose)
[3] Parasite Clearance Time, i.e. time between drug intake and first negative thick blood smear
[4] Fever Clearance Time, i.e. time between drug intake and normalization of body temperature ($\leq$ 37.5°C)

4.3 Pharmacokinetics of Mefloquine

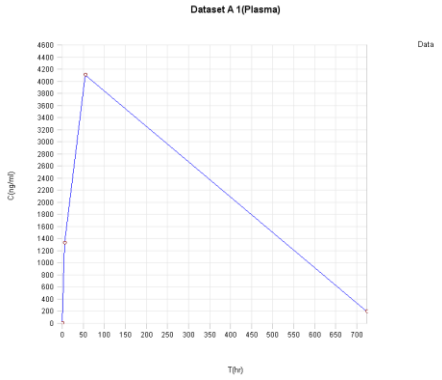
Drug concentrations of mefloquine were comparable in the two treatment groups at the respective time points. 6 hours after first drug administration median plasma levels were 714 ng/ml in treatment group A and 588 ng/ml in group B. There was a trend towards higher mefloquine concentrations 54 hours after initiation of treatment in the younger age group receiving the fixed-dose paediatric drug formulation (median plasma levels 2550 ng/ml and 1815 ng/ml respectively, p=0.10). At the end of the observation period on day 28 mefloquine levels in group B were slightly higher than in group A (median plasma levels 197 ng/ml in A and 343 in B, p=0.07). Table 8 summarizes mefloquine plasma concentrations in the respective age groups.

Table 8 Mefloquine plasma levels

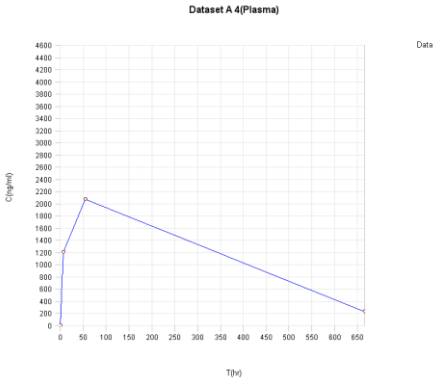
	Group A (paediatric formulation)				Group B (co-blister tablets)			
	Mean [90%CI]	Median	min	max	Mean [90%CI]	Median	min	max
Conc. 6h (ng/ml)	706 [522-891]	724	85	1330	620 [461-778]	588	287	1380
Conc. 54h (ng/ml)	2578 [2021-3134]	2550	810	4460	1907 [1585-2230]	1815	859	2950
Conc. 28d (ng/ml)	239 [170-308]	197	100	518	337 [276-398]	343	343	544

The following figures (figures 8-14) show mefloquine plasma concentration versus time curves for the individual patients and mean plasma concentration/time profiles for the 2 treatment groups:

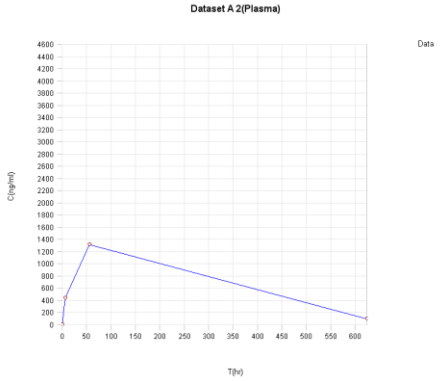
Figure 8 Mefloquine plasma concentrations of patients A1-A6



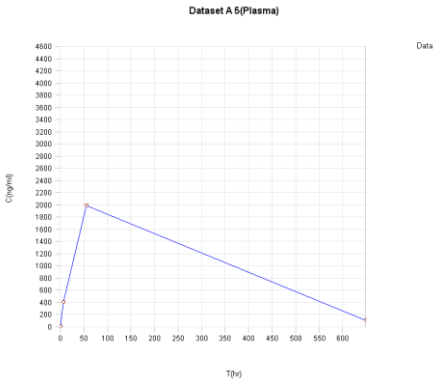
gender	m	C_6 (ng/ml)	1330
age (ys)	5	C_{54} (ng/ml)	4100
weight (kg)	15	C_{650} (ng/ml)	192
height (cm)	101	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	1.43



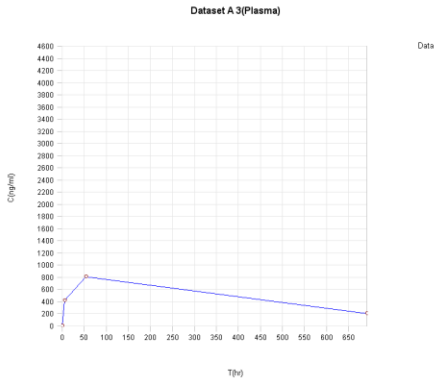
gender	f	C_6 (ng/ml)	1210
age (ys)	4	C_{54} (ng/ml)	2080
weight (kg)	14.5	C_{650} (ng/ml)	235
height (cm)	98	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.71



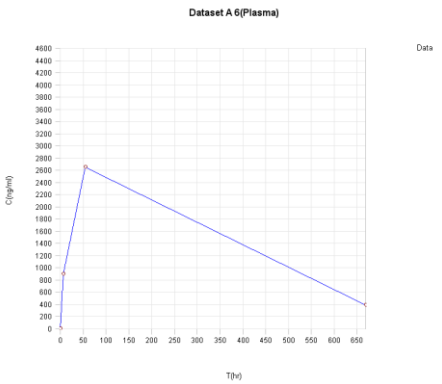
gender	m	C_6 (ng/ml)	441
age (ys)	3	C_{54} (ng/ml)	1320
weight (kg)	16	C_{650} (ng/ml)	100
height (cm)	99	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.40



gender	m	C_6 (ng/ml)	400
age (ys)	5	C_{54} (ng/ml)	1990
weight (kg)	16	C_{650} (ng/ml)	107
height (cm)	104	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.62

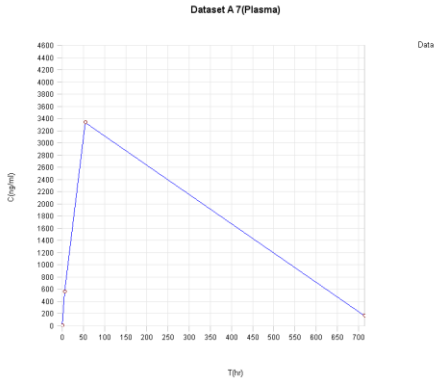


gender	f	C_6 (ng/ml)	416
age (ys)	6	C_{54} (ng/ml)	810
weight (kg)	16	C_{650} (ng/ml)	202
height (cm)	106	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.32

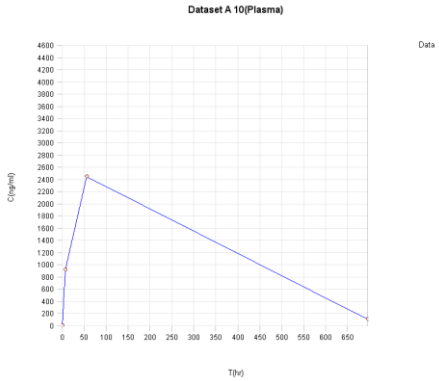


gender	f	C_6 (ng/ml)	899
age (ys)	6	C_{54} (ng/ml)	2650
weight (kg)	19	C_{650} (ng/ml)	390
height (cm)	113	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.93

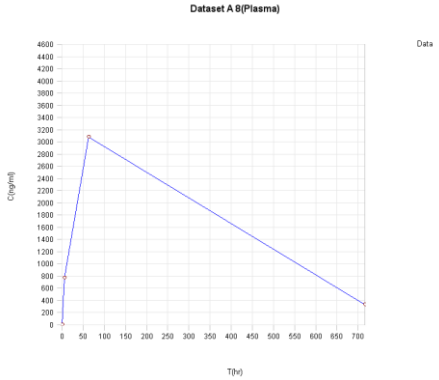
Figure 9 Mefloquine plasma concentrations of patients A7-A12



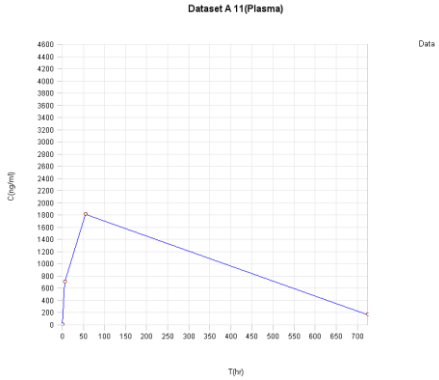
gender	f	C_6 (ng/ml)	554
age (ys)	5	C_{54} (ng/ml)	3330
weight (kg)	15	C_{650} (ng/ml)	161
height (cm)	105	AUC_{0-t} (10^6h*ng/ml)	1.15



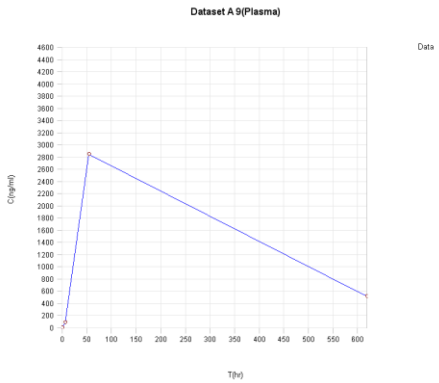
gender	m	C_6 (ng/ml)	919
age (ys)	6	C_{54} (ng/ml)	2450
weight (kg)	18	C_{650} (ng/ml)	108
height (cm)	113	AUC_{0-t} (10^6h*ng/ml)	0.82



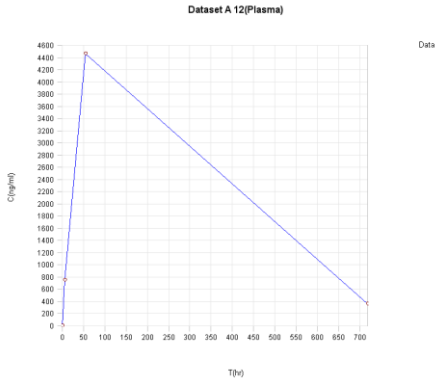
gender	f	C_6 (ng/ml)	774
age (ys)	5	C_{54} (ng/ml)	3080
weight (kg)	12	$AUC_{0-\infty}$ (h*ng/ml)	332
height (cm)	104	AUC_{0-t} (10^6h*ng/ml)	1.11



gender	f	C_6 (ng/ml)	697
age (ys)	6	C_{54} (ng/ml)	1810
weight (kg)	14	C_{650} (ng/ml)	163
height (cm)	105	AUC_{0-t} (10^6h*ng/ml)	0.66

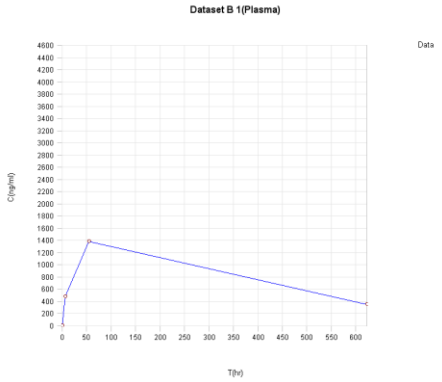


gender	m	C_6 (ng/ml)	86
age (ys)	3	C_{54} (ng/ml)	2850
weight (kg)	16	C_{650} (ng/ml)	518
height (cm)	100	AUC_{0-t} (10^6h*ng/ml)	0.95

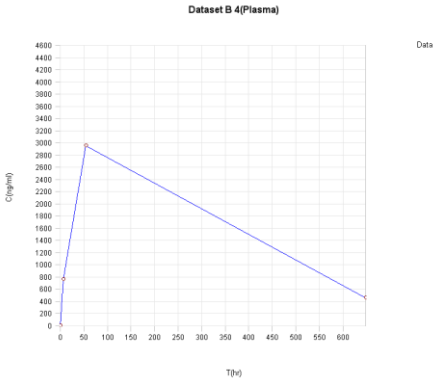


gender	f	C_6 (ng/ml)	751
age (ys)	2	C_{54} (ng/ml)	4460
weight (kg)	11.2	C_{650} (ng/ml)	363
height (cm)	90	AUC_{0-t} (10^6h*ng/ml)	1.60

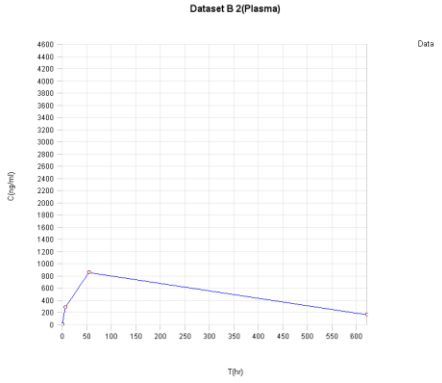
Figure 10 Mefloquine plasma concentrations of patients B1-B6



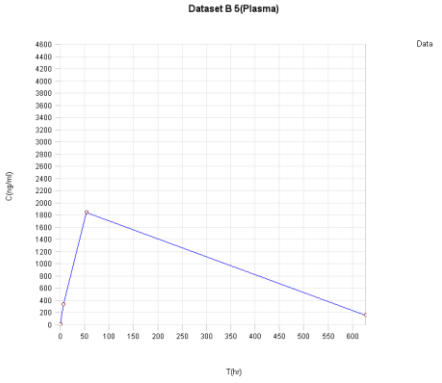
gender	f	C_6 (ng/ml)	474
age (ys)	10	C_{54} (ng/ml)	1380
weight (kg)	24	C_{650} (ng/ml)	349
height (cm)	120	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.49



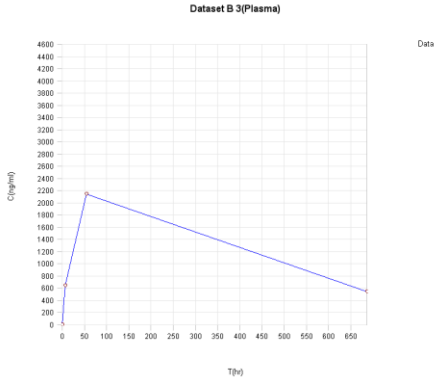
gender	m	C_6 (ng/ml)	765
age (ys)	11	C_{54} (ng/ml)	2950
weight (kg)	23	C_{650} (ng/ml)	455
height (cm)	129	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	1.01



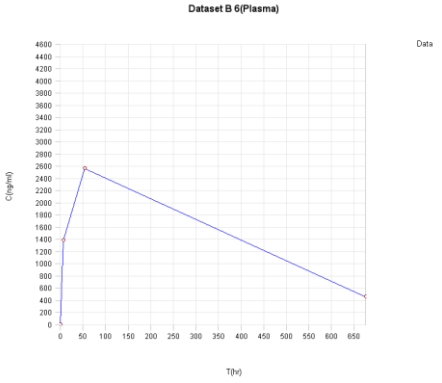
gender	m	C_6 (ng/ml)	287
age (ys)	11	C_{54} (ng/ml)	859
weight (kg)	35	C_{650} (ng/ml)	164
height (cm)	152	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.29



gender	f	C_6 (ng/ml)	332
age (ys)	12	C_{54} (ng/ml)	1840
weight (kg)	31	C_{650} (ng/ml)	156
height (cm)	130	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.57

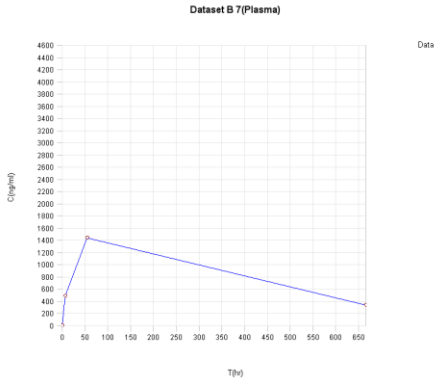


gender	m	C_6 (ng/ml)	641
age (ys)	6	C_{54} (ng/ml)	2140
weight (kg)	23	C_{650} (ng/ml)	544
height (cm)	123	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.84

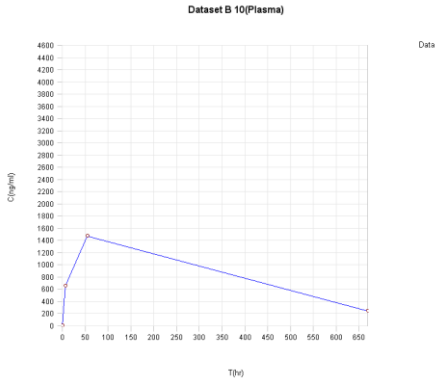


gender	f	C_6 (ng/ml)	1380
age (ys)	7	C_{54} (ng/ml)	2560
weight (kg)	21	C_{650} (ng/ml)	461
height (cm)	120	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.94

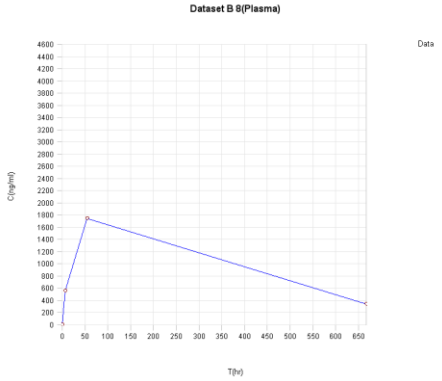
Figure 11 Mefloquine plasma concentrations of patients B7-B12



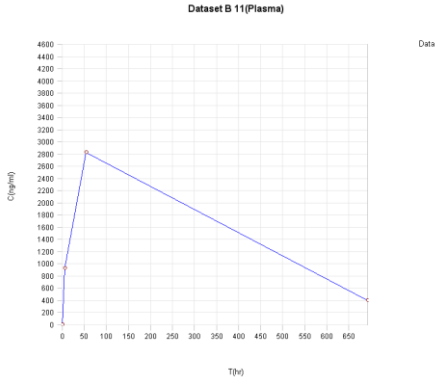
gender	m	C_6 (ng/ml)	491
age (ys)	11	C_{54} (ng/ml)	1440
weight (kg)	30	C_{650} (ng/ml)	342
height (cm)	141	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.54



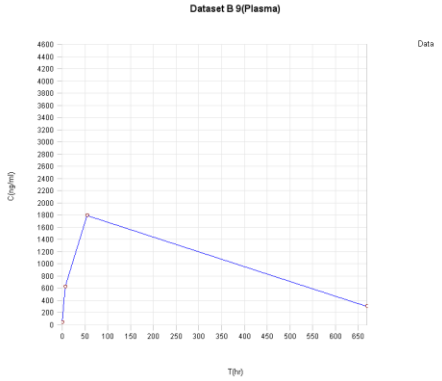
gender	m	C_6 (ng/ml)	650
age (ys)	9	C_{54} (ng/ml)	1470
weight (kg)	30	C_{650} (ng/ml)	239
height (cm)	140	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.52



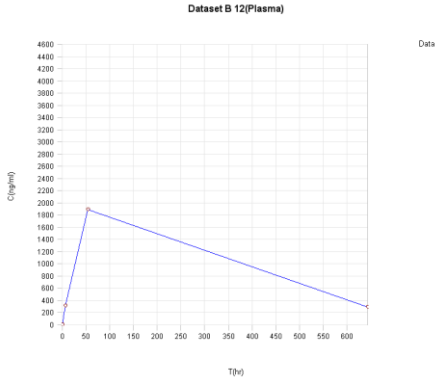
gender	f	C_6 (ng/ml)	551
age (ys)	10	C_{54} (ng/ml)	1740
weight (kg)	24	C_{650} (ng/ml)	343
height (cm)	125	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.64



gender	m	C_6 (ng/ml)	650
age (ys)	8	C_{54} (ng/ml)	1470
weight (kg)	24	C_{650} (ng/ml)	239
height (cm)	128	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	1.03



gender	m	C_6 (ng/ml)	625
age (ys)	11	C_{54} (ng/ml)	1790
weight (kg)	37	C_{650} (ng/ml)	305
height (cm)	152	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.64



gender	m	C_6 (ng/ml)	311
age (ys)	12	C_{54} (ng/ml)	1890
weight (kg)	36	C_{650} (ng/ml)	289
height (cm)	155	$AUC_{0\text{-}t}$ (10^6h*ng/ml)	0.64

Figure 12 Mefloquine plasma concentrations for patients of group A (fixed-dose formulation)

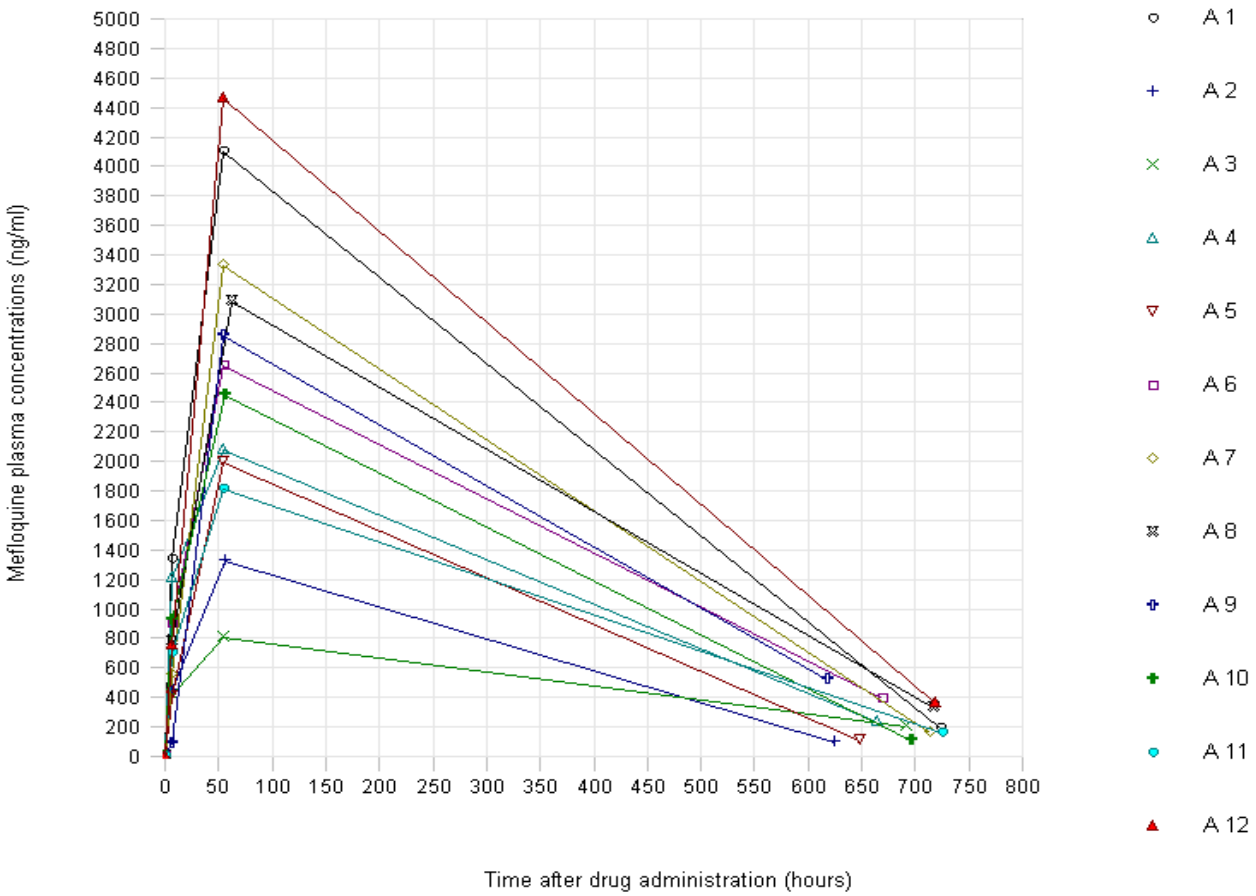


Figure 13 Mefloquine plasma concentrations for patients of group B (co-blister tablets)

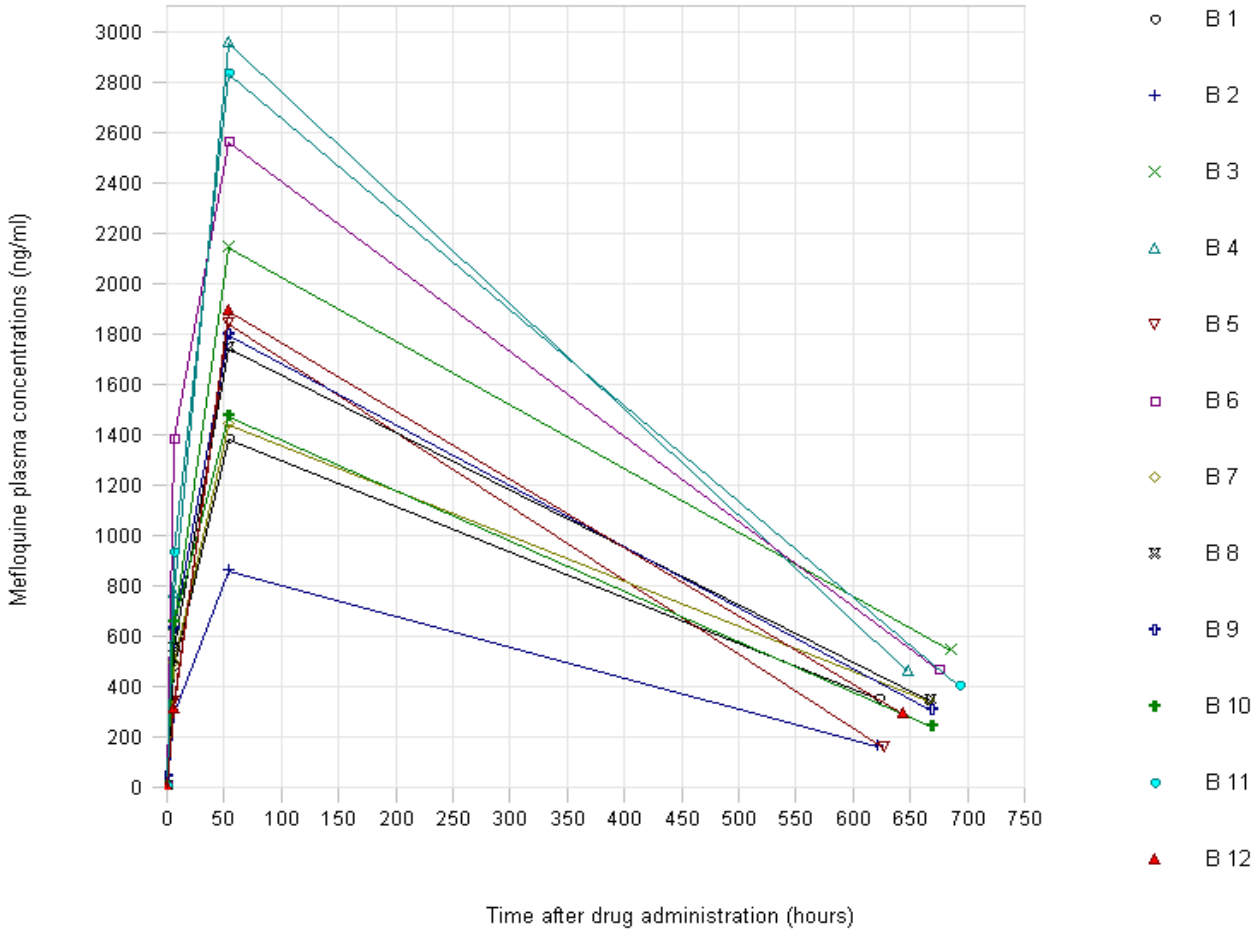
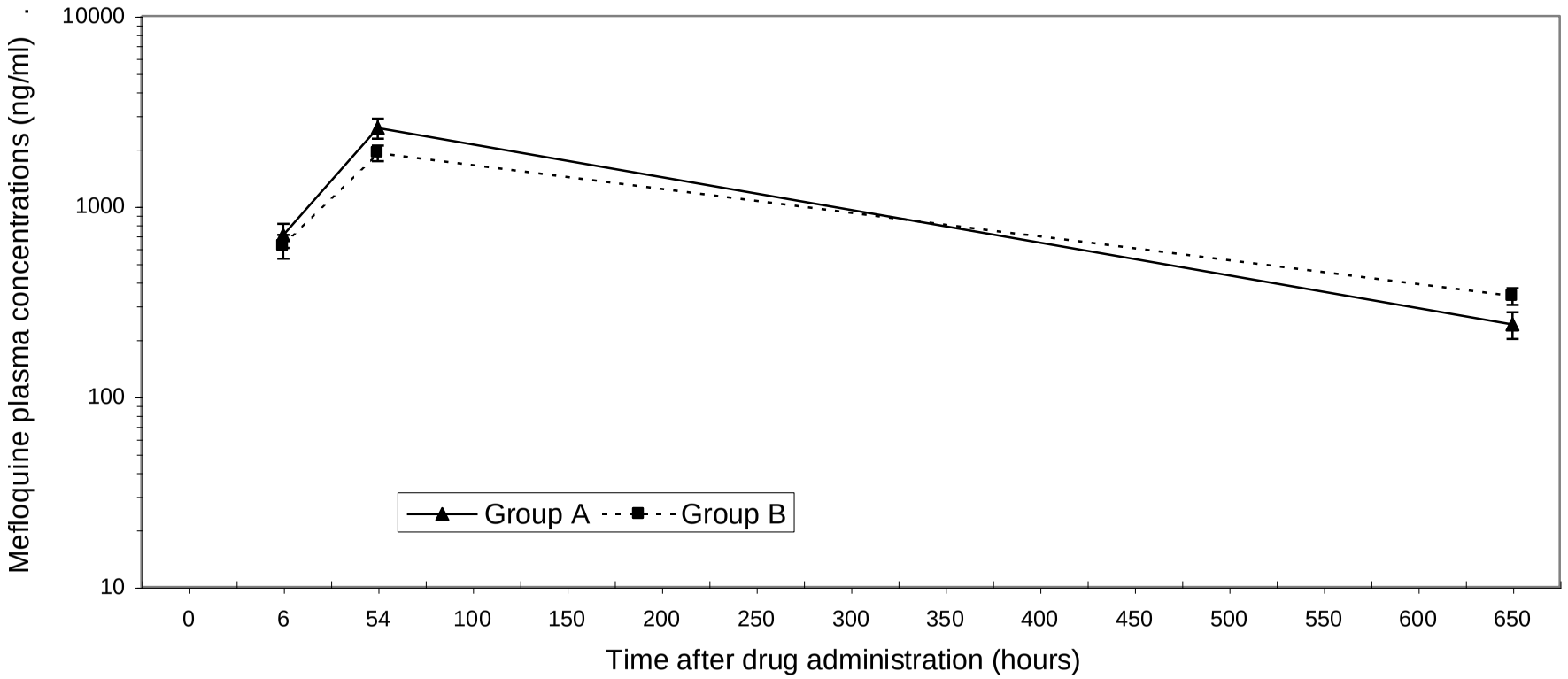


Figure 14 Mean plasma-concentration versus time profile curve of mefloquine for treatment groups A (fixed-dose formulation) and B (co-blister tablets)



Time points for plasma sampling are depicted on x-axis.

Log transformed mean plasma concentrations and standard error of the mean are depicted on y-axis

5 Discussion

Children under 5 are the group at highest risk for malaria related morbidity and mortality. They account for around 90% of more than 1 million malaria deaths each year [91]. Nevertheless there is a lack of adequate paediatric drug formulations for most current antimalarial treatment combinations.

Artemisinin derivatives are a major advance in the fight against the enormous disease burden caused by malaria infections. Generic formulations of artemisinin containing combinations can help to make these new medicines affordable and available for all people in need. However, several trials evaluating generic products of oral artesunate and mefloquine highlighted the importance of their galenic form and its impact on pharmacokinetic characteristics: Different formulations might for example vary in their bioavailability, possibly leading to insufficient blood levels and therefore impairing the medicaments' therapeutic power [92-94].

The worldwide emergence of multidrug resistance has lead to an urgent need for new antimalarial drugs being effective and safe, specially evaluated in the respective target population, produced according to international Good Manufacturing Practice (GMP) standards, available as specific formulations for paediatric patients and developed for unpretentious administration in home based treatment settings.

The present study assessed pharmacokinetic properties of two oral paediatric formulations of artesunate and mefloquine in two groups of African children suffering from uncomplicated *Plasmodium falciparum* malaria.

Both formulations were readily absorbed and yielded satisfying therapeutic plasma concentrations of mefloquine and dihydroartemisinin, the active metabolite of artesunate. Despite considerable variations in drug plasma concentrations, significant antimalarial activity was attained in all children, leading to quick clinical and parasitological responses.

Three patients in the younger age group who were treated with the fixed-dose stick-pack formulation exhibited a pattern of delayed and comparably low dihydroartemisinin peak plasma levels. Plasma concentration versus time curves were unequal in all three cases: dihydroartemisinin values of patient A5 reached a plateau around 150 ng/ml 2 hours after drug administration with the maximum concentration after 6 hours (158 ng/ml). Plasma levels of patient A 7 were more or less constantly rising until the maximum of 420 ng/ml after 6 hours. Patient A 9 showed dihydroartemisinin levels just little above or below the detection limit (10 ng/ml) up to 4 hours after drug administration, then reaching a value of 130 ng/ml at 6 hours. For all three patients it is likely that C_{max} was not adequately captured by the 6 hours sampling scheme.

As in the three patients, lower C_{max} were generally associated with longer T_{max} for the whole study population ($r=0.57$, $p=0.004$). On the one hand this may be attributable to the sampling schedule: whereas blood samples were taken every 30 minutes during the first two hours, intervals between blood draws change to 120 minutes afterwards. This makes it much more likely for plasma peaks later than two hours to be missed, as there is possibly no blood draw close enough to it. In our study, 6 out of 7 patients who had C_{max} levels lower than 500 ng/ml hit these values at a time point 2 hours or longer after drug intake, thus in this period of enhanced intervals.

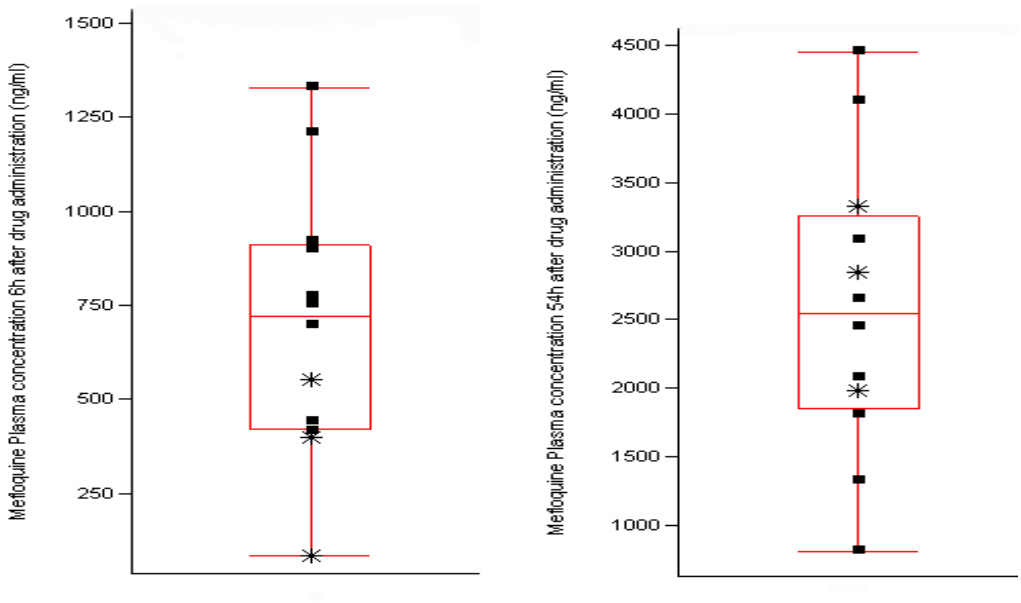
On the other hand there might have been metabolic differences in certain patients, affecting absorption of artesunate and its transformation to dihydroartemisinin. Artesunate is hydrolysed to dihydroartemisinin in the gastrointestinal tract at highly variable rates; its stability is pH-dependent with short half-life in acid milieu (10.3 min at pH 1.2) and significantly longer $t_{1/2}$ at neutral pH (7.3 hours in plasma)[53]. More acidity and longer duration in the stomach leads to more hydroxylation of artesunate to dihydroartemisinin before absorption. Thus a relatively higher proportion of drug is absorbed in the form of dihydroartemisinin, leading to faster rise of dihydroartemisinin plasma levels. On the contrary, higher pH in stomach means higher stability of artesunate in the gastrointestinal tract, therefore a higher artesunate/dihydroartemisinin ratio

during absorption and slower ascent of dihydroartemisinin concentration-time curve in plasma.

C_{max} of dihydroartemisinin was positively correlated with mefloquine levels 6 hours after drug intake (r=0.53, p=0.007). For later time points of mefloquine sampling (54h, d28) there was no correlation with dihydroartemisinin plasma levels. Thus patients with lower dihydroartemisinin peak plasma levels also tended to show diminished initial mefloquine absorption, but attained average mefloquine plasma concentrations thereafter. According to this, the three patients in group A who exhibited delayed absorption of artesunate (A5, A7, A9) had comparably low mefloquine plasma levels 6 hours after drug administration (A5: 400 ng/ml, A7: 554 ng/ml, A7: 86 ng/ml, median concentration in group A: 724 ng/ml). After 54 hours mefloquine concentrations of these patients were well within the normal range of this treatment group (A5: 1990 ng/ml, A7: 3330 ng/ml, A9: 2850 ng/ml, median mefloquine concentration in group A at 54 hours: 2550 ng/ml). Figure 15 demonstrates the distribution of mefloquine plasma concentrations of the individual patients in group A 6 and 56 hours after drug administration. The 3 patients with lowest rate of dihydroartemisinin absorption (A5, A7, A9) are marked by starlets (*) in the two graphs, respectively.

Mefloquine absorption is known to be augmented by administering the drug together with food, thus also connected to stimulated gastric acid secretion and longer duration in the stomach [95]. Food intake – or fasting respectively - could therefore be a possible explanation for both, quicker appearance of dihydroartemisinin in plasma and better absorption of mefloquine in the initial phase after treatment - or the opposite, slower rise of dihydroartemisinin plasma levels and lower mefloquine plasma levels. Documentation of food intake and detection of the parent compound artesunate in plasma, both not performed in our study, could reveal further details about this coherence.

Figure 15 Distribution of mefloquine plasma concentrations of patients in treatment group A 6 hours and 54 hours after treatment



Patients with delayed absorption of artesunate (*) had comparably low mefloquine plasma levels 6 hours after drug intake, but reached normal levels after 54 hours

Comparison with previous reports on pharmacokinetics of oral artemisinins

To date there have been no reports on pharmacokinetic characteristics of oral administered artemisinins in African children. Results from the present study may therefore be of value to enlarge our pharmacological knowledge of these compounds for an important group of patients.

Previously published data on pharmacokinetics of oral artesunate are mostly restricted to studies in healthy volunteers [96-101]. Yet it has become clear that malaria infection impairs distribution and metabolism of artemisinin antimalarials. Clinical studies as well as findings in the *Plasmodium berghei* rodent malaria model suggest reduced hepatic clearance of dihydroartemisinin in patients with malaria, leading to higher peak plasma levels and augmented bioavailability during disease [51, 52, 102, 103]. Moreover, it seems that malaria lowers the apparent volume of distribution of dihydroartemisinin [51, 52]. In children absorption, distribution, metabolism and elimination of drugs can generally be different from adults [104].

Bethell *et al.* [39] studied the pharmacokinetic profile of oral artesunate in 10 children from Vietnam, reporting results for absorption (mean T_{max} = 1.7h) and elimination (mean $t_{1/2}$ = 1.0h), which are consistent with the present study. A different model-independent methodology was employed in order to calculate pharmacokinetic parameters. Dihydroartemisinin plasma levels were determined by a radioisotopic bioassay, detecting overall antimalarial activity in plasma instead of single metabolites [105]. Nonetheless maximum plasma activity and AUC_{0-t} values after 3 mg/kg doses are comparable to our trial (mean C_{max} =664 ng/ml and mean AUC_{0-t}=1286 h*ng/ml) with similar findings of high interindividual variation (C_{max} range=179-1395 ng/ml, AUC_{0-t} range=608-2258 h*ng/ml).

In a study by Binh and colleagues [51], pharmacokinetics of 150 mg oral artesunate (approx. 2.8 mg/kg) were determined in 8 adult Vietnamese patients. High Performance Liquid Chromatography with UV-detection was used for drug determination and pharmacokinetic descriptors were obtained from non-compartmental analysis as in our study. The reported values for C_{max}, T_{max} , $t_{1/2}$ and $AUC_{0-\infty}$ were 1308 ng/ml (95%CI 882-1735), 1.58 h (IQR 0.83-2.0), 0.88 h (95%CI 0.65-1.12) and 2531 h*ng/ml (95%CI 1678-3384), respectively, and are consistent with data from the current study. The shorter terminal half-life is in line with other results from the same group [106], reporting a mean $t_{1/2}$ of 0.66h (95%CI 0.53-0.78) for dihydroartemisinin after 100 mg oral artesunate in adult Vietnamese patients. Given that hepatic clearance of drugs in children is normally faster than in adults [104], the comparably longer half-life in our patients indicates a tendency towards slower elimination of artemisinin drugs in African children with malaria. This could be of importance in young patients suffering from severe malaria, when hepatic metabolism is additionally impaired.

The following table shows data from previous reports on pharmacokinetics of dihydroartemisinin in comparison with data from our study.

Table 9 Reports on dihydroartemisinin pharmacokinetic parameters after administration of oral artesunate

Study	Method	Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (h*ng/ml)
Group A *	HPLC	3.3	861 (130-3390) ‡	1.5 (1.0-6.1) ‡	0.9 (0.6-2.2) ‡	3024 (1399-6679) ‡
Group B *	HPLC	3.7	930 (286-2190) ‡	1.5 (0.5-4.1) ‡	1.0 (0.6-10.1) ‡	2815 (1049-7791) ‡
Bethell [39]*	BA	3	664 (179-1395) ¥	1.7 (0.25-4.0) ¥	1.0 (0.54-2.31) ¥	1286 (608-2258) ¥
Binh [51]	HPLC	2.8	1308 (882-1735) †	1.58 (0.83-2.0)§	0.88 (0.65-1.12) †	2531 (1678-3383) †
Batty [106]	HPLC	2.5	737 (355-2349) §	1.5 (1-2.25)§	0.66 (0.53-0.78) †	1288 (759-3097) §
T.-Isavadharm [103]	HPLC	1.8	554 (250-808) ¥	1.5 (1.0-2.0) ¥	0.80 (0.68-1.38) ¥	1144 (516-1850) ¥
Suputtamongkol [107]	BA	4.0	2300(1298-7003) ‡	1.5 (0.5-4.0) ‡	1.31 (0.55-4.71) ‡	5365 (4592-6138) ‡

molar weight of 284.4 for Dihydroartemisinin was used for conversion
* studies with paediatric patients
‡ median (range)
¥ mean (range)
† mean (95% CI)
§ median (interquartile range)

Comparison of pharmacokinetic data of rectal artesunate-formulations

In addition to oral formulations of artesunate, its rectal use has widely been advocated as promising treatment of paediatric malaria [108]. Especially in rural settings, suppositories can be a valuable alternative to intravenous treatment for children who are unable to take oral medication. Artesunate is the only artemisinin derivative that is water soluble and therefore easily absorbable across the rectal mucosa. Several studies have been conducted in order to evaluate the rectal use of artesunate in children [109-113]. In general absorption of artesunate and transformation to dihydroartemisinin after rectal administration is slower compared to oral formulations (time to peak values between 2 and 3 hours). All studies reported substantial differences in bioavailability and high patient to patient variability, yet clinical courses and parasitological responses were satisfactory. Interestingly, even children who seemingly expelled suppositories and therefore showed no changes in dihydroartemisinin plasma levels after certain drug administrations, overall responded well to treatment.

Artesunate dose response relationship

The clinical significance of dihydroartemisinin plasma concentrations with respect to clinical and parasitological efficacy parameters is complex and, to date, not fully understood. It is not known which pharmacokinetic parameter is the most important determinant and to what extent the peak concentration, the area under the plasma-concentration time curve or time above a certain minimum inhibitory concentration have an influence on pharmacodynamic effects. Brian Angus and colleagues [114] assessed the dose response relationship of oral artesunate in acute *Plasmodium falciparum* malaria and reported that an increase of doses above the level of 2 mg/kg had no additional effect in terms of faster parasite reduction in average adults. In our study all children exhibited fast parasitological and clinical responses, despite considerable differences in blood concentration time profiles. Higher dihydroartemisinin plasma levels had only little effect on the clinical course of

the disease. In one group patients with higher plasma levels had shorter fever clearance times, in the second group higher peak plasma levels were related to higher parasite reduction rates during the first 6 hours after drug intake. Yet, no influence neither on overall parasite clearance times nor on parasite reduction rates after 12 hours could be found.

Concerning parasiticidal effects of antimalarials in general, estimates on necessary plasma concentrations can be derived from *in vitro* growth inhibition assays. Thereby 99% *in vitro* growth inhibition of an antimalarial is considered as threshold for minimum inhibitory concentrations (MIC) in non-immune patients. Previous results from *in vitro* drug sensitivity studies in Gabon found average dihydroartemisinin concentration of 9.7 ng/ml as minimum inhibitory concentration in non-immune patients [88]. This threshold was surpassed widely even by the lowest observed C_{max} level in our study (130ng/ml).

In conclusion artemisinins can be administered in dosages which securely lead to dihydroartemisinin plasma levels well above the threshold of parasites' sensitivity to the drug, even though dose plasma responses are highly variable. This fact is mainly owed to the low toxicity profiles and the high therapeutic indices of artemisinin derivatives and contributes importantly to their sustained high efficacy.

Mefloquine plasma concentrations

Among the 2 compounds of the combination treatment, artesunate causes rapid elimination of parasites and quick relief of clinical symptoms. Artemisinins can reduce the infecting biomass by a factor of approximately 10^{-4} per life cycle, which means killing 99.99% of parasites - more than any other antimalarial drug [115]. With a 3 day treatment course, artesunate is present during 2 parasite life cycles, which would correspond to parasite reduction by a factor of 10^{-8}. In order to ensure complete cure of the patient, the remaining parasites have to be removed by the partner drug mefloquine. It should therefore be present in blood at parasiticidal levels from the moment when

activity of dihydroartemisinin declines until the last parasite has been eliminated.

For ethical reasons the sampling schedule for mefloquine plasma levels in our study was restricted to 3 time points: 6 hours after the first dose on day 1, 6 hours after the last dose on day 3 and 4 weeks after treatment on day 28. Previous studies on pharmacokinetics of mefloquine found time to peak concentration values between 10 and 20 hours after drug administration [34, 66, 67]. It is therefore likely that our samplings failed to capture the maximum plasma concentration, and blood levels on day 3 are probably close to but still underestimates of true C_{max} values. The observed plasma levels on day 3 were, however, within the range of previously reported C_{max} values after 25 mg/kg doses in children from Thailand [67].

Comparison between the two groups reveals similar plasma concentrations 6 hours after the first dose on day 1. On day 3 children in group A had higher median mefloquine plasma levels than children in group B (2550 and 1815 ng/ml, respectively). By contrast, median concentrations on day 28 were higher in group B (343 ng/ml) than in group A (197 ng/ml).

Despite restrictions due to inaccurate sampling times and differences in drug formulation, these findings indicate faster mefloquine elimination from plasma in younger children.

Previous studies found mefloquine plasma concentrations to be predictive for treatment outcome. In a study by Slutsker *et al*. [116] in children from Malawi, mefloquine blood concentrations lower than 500 ng/ml on day 2 and 7 after treatment were strongly associated with parasitological treatment failure on day 7. In this study, among 13 children who exhibited mefloquine plasma levels lower than 500 ng/ml on the second day after treatment, 6 (46%) were parasitaemic on day 7. In contrast only 2 (3%) of 58 children with mefloquine concentrations above 500 ng/ml on day 2 were parasitaemic on day 7 ($p< 0.0003$).

In our study all children had mefloquine plasma concentrations above 500 ng/ml on day 3. Hypothesising linear regression between day 3 and day 28, plasma levels remained above 500 ng/ml for 21.8 and 22.3 days in treatment group A and B, respectively. For the 2 patients who showed the lowest plasma levels in their respective group on day 3 (patient A3: 810 ng/ml, patient B2: 859 ng/ml), linear regression analysis indicated mefloquine levels above 500 ng/ml for 12.7 and 12.9 days, respectively.

In vitro drug sensitivity monitoring from our study site found mefloquine levels of 1550 ng/ml (4.1 µmol/l) to exhibit 99% growth inhibition of *Plasmodium falciparum* [85]. Postulating *in vitro* EC 99 concentrations to be equivalent to minimum inhibitory concentrations (MIC) *in vivo*, median mefloquine plasma levels stayed above this threshold for 11.1 and 5.3 days in group A and B, respectively.

Mefloquine blood levels are not only important for clearance of the current infection but will also provide protection against re-infection for a certain period after treatment. In general long acting antimalarials may reduce the overall incidence of malaria after infection, a fact that has lately been emphasized by some authors [45]. Yet at the same time the prolonged activity in plasma increases the risk to select for new parasites with decreased susceptibility for the drug given [117].

In Thailand and Cambodia, the potential of artesunate-mefloquine to select for parasites with increased copy numbers of the *Plasmodium falciparum multi drug resistance gene 1 (pfmdr-1)* has recently been highlighted [118]. This amplification of the *pfmdr1*-gene is the most important determinant of *in vitro* and *in vivo* resistance to mefloquine and strongly associated with treatment failure after artesunate-mefloquine administration [118, 119]. Parasites with amplified *pfmdr1* genes have also been identified in Gabon [120].

The reciprocative protection of co-administered substances against new drug resistant parasites is one of the major advantages of combination therapies.

After the elimination of dihydroartemisinin form plasma, its partner, in our case mefloquine, remains unprotected in blood for a considerable period. Implications of this exposure of sub-therapeutic blood levels to possible new-infections, especially in areas with intense transmission and therefore frequent occurrence of re-infection early after previous malaria episodes, need to be seen in continued monitoring of drug resistance.

6 Conclusion

In this study we assessed the pharmacokinetic characteristics of two oral formulations of artesunate in combination with mefloquine in African children suffering from acute, uncomplicated *Plasmodium falciparum* malaria. Patients included in this analysis were a sub-group of children participating in a trial assessing efficacy, safety, tolerability and acceptability of the investigational drugs.

A group of 12 children between 10 and 20 kg bodyweight were treated with a novel fixed-dose co-formulation of 50 mg artesunate and 125 mg mefloquine daily for three consecutive days. Median peak dihydroartemisinin concentration in plasma was 861 ng/ml, the area under the concentration time curve 3024 h*ng/ml. A second group of 12 children between 20 and 40 kg bodyweight received 100 mg artesunate and 250 mg mefloquine as tablets for three days. Median C_{max} in this group was 930 ng/ml with a median $AUC_{0-\infty}$ of 2815 h*ng/ml. Both formulations were rapidly absorbed (median T_{max} of 1.5h in both groups). Plasma elimination half-lives of dihydroartemisinin were 0.9 hours in the younger and 1.0 hours in the older group. As in previous studies, reporting on pharmacokinetics of artemisinin compounds, there was marked inter-individual variation in plasma concentration versus time curves.

Analysis of mefloquine plasma levels revealed a trend towards higher concentrations in the younger age group during the absorption phase (2550 and 1815 ng/ml 54 hours after initiation of treatment, respectively). Median mefloquine concentrations at day 28 were 197 and 343 ng/ml, respectively.

Overall, the pharmacokinetic characteristics of the two paediatric dosage forms, i.e. the novel fixed-dose co-formulation and the standard co-blister were comparable in the two respective groups. All children attained sufficient blood levels for treatment of *Plasmodium falciparum* malaria in the respective area.

Development of drug formulations for African children, the group at highest risk of malaria associated morbidity and mortality, is of high importance. In this respect, the new fixed-dose formulation is an interesting new option for

treatment on outpatient basis. To what extent the combination of artesunate and mefloquine will be employed in Sub-Saharan Africa will depend on a number of factors, including its price and the potential for the development of resistance to either component. Epidemiological evidence for the usefulness of artesunate-mefloquine in Africa as well as large clinical trials evaluating the safety and tolerability of the novel drug formulations for the oral treatment of African children are now needed as scientific basis for their recommendation and wide scale use.

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9 Curriculum Vitae

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