INVESTIGATION OF BIOAVAILABILITY, PHARMACOKINETICS AND SAFETY OF NEW PEDIATRIC FORMULATIONS OF ARTESUNATE AND MEFLOQUINE

L Chanthap[1], R Tsuyuoka[2], K Na-Bangchang[3], N Nivanna[4], D Suksom[3], T Sovannarith[4] and D Socheat[1]

[1]National Center for Parasitological Entomology and Malaria Control Program, Ministry of Health, Cambodia; [2]World Health Organization, Cambodia; [3]Faculty of Allied Health Sciences, Thammasat University (Rangsit Campus), Pathum Thani, Thailand; [4]National Laboratory for Drug Quality Control, Ministry of Health, Cambodia

Abstract. The bioavailability/pharmacokinetics of dihydroartemisinin and mefloquine following the oral doses of 4 mg/kg body weight artesunate (Cambodian Pharmaceutical Enterprise) given concurrently with 10 mg/kg body weight oral mefloquine artesunate (Cambodian Pharmaceutical Enterprise) were investigated in 15 healthy Cambodian male volunteers. Both formulations were generally well tolerated. Both produced satisfactory plasma/blood concentration-time profiles. Oral artesunate and mefloquine were rapidly absorbed from gastrointestinal tract with marked inter-individual variation. For the dihydroartemisinin, the median (95% CI) C_{max} of 748 (304-1,470) ng/ml was observed at 1.5 (0.3-3.0) hours (t_{max}) after drug administration. The median (95% CI) values for $AUC_{0-\infty,}$ λ_z and $t_{1/2z}$ were 1.673 (1.08-2.88) μg.h/ml, 0.54(0.24-1.1)/hour and 1.3 (0.6-2.9) hours, respectively. For mefloquine, a median (95% CI) C_{max} of 1,000 (591-1,500) ng/ml was observed at 4 (2-6) hours (t_{max}) after drug administration. The median (95% CI) value for AUC_{0-168h} was 3.92 (2.88-7.02) μg.h/ml.

INTRODUCTION

Malaria is a leading cause of mortality and morbidity in developing areas of the world, and remains a major public health problem in endemic regions (Berman *et al,* 2001). Resistance to available drugs is increasing, creating a need for new drugs that are well tolerated and simple to use. In the face of this ominous situation, artemisinin and its derivatives (artesunate, artemether, arteether, and dihydroartemisinin) have given renewed hope for combating resistant malaria (Hein, 1993; Harinasuta and Karbwang, 1994). These drugs have gained considerable prominence in the chemotherapy of both uncomplicated and severe falciparum malaria by demonstrating high activity against multidrug-resistant falciparum strains with low toxicity profiles.

Correspondence: Dr Kesara Na-Bangchang, Pharmacology of Infectious Diseases Research and Service Unit, Faculty of Allied Health Sciences, Thammasat University, Rangsit Campus, Paholyothin Road, Klong Luang, Pathum Thani 12121, Thailand.
Fax: +66 (0) 2986 9209
E-mail: kesaratmu@yahoo.com

Due to the high recrudescence rates from the mono-therapeutic regimens of artemisinin derivatives (Harinasuta and Karbwang, 1994), the use of these drugs in combination with drugs with long half-lives, *eg* mefloquine, has been increasingly advocated for achievement of radical cure. The combination of artemisinin derivatives, especially artesunate, with mefloquine is commonly applied in areas with marked mefloquine resistance. There are potential advantages of combining artemisinin derivatives with mefloquine such as reducing the needed dose and treatment period, which should improve compliance. The combination of artesunate and mefloquine is associated with a more rapid treatment response, improved cure rate and slow development of mefloquine resistance (Price *et al*, 1996; White, 1997). Short course combination (2-days) regimens of artesunate with mefloquine have proved effective with good patient compliance (Bunnag *et al*, 1996).

The usual combination is 3 days of artesunate (3-4 mg/kg body weight) and mefloquine (25 mg/kg body weight) in single or split doses of 15 followed by 10 mg/kg body weight, with

cure rates approaching 100% in both adults and children with uncomplicated falciparum malaria (Thimasarn *et al,* 1997; Price *et al,* 1998; Sabcharoen *et al,* 1998; McGready *et al*, 2000). Since 1999, the first-line national guideline policy of Cambodia for the treatment of uncomplicated falciparum malaria is the combination of artesunate and mefloquine. To facilitate the compliance of drug intake in children with malaria, new pediatric formulations of artesunate (45 and 65 mg) and mefloquine (65 and 100 mg) were developed in Cambodia and registered with the Department of Drug and Food, Cambodian Ministry of Health.

It is well recognized that generic formulations with identical contents of active substances may show different physicochemical properties, which may influence the absorption and disposition of the active substance. Alterations of the rates and extents of absorption can be clinically relevant for agents such as antimalarials when a prompt and effective response is desired. A lack of bioequivalence of generic mefloquine and artesunate tablets has been reported (Na-Bangchang *et al*, 1998a, 2000; Weidekamm *et al*, 1998). Since, adequate blood/plasma concentrations attained during the acute phase of malaria infection is essential and prerequisite for the elimination of malaria parasites, investigation of the bioavailability of the oral formulations of these drugs, including the active metabolites, is necessary. The objective of the present study was to describe the bioavailability/pharmacokinetics of the new pediatric oral formulations of artesunate and mefloquine produced by Cambodian Pharmaceutical Enterprise, Cambodia.

MATERIALS AND METHODS

Subjects

The study was conducted in December, 2002 at Bayon Hospital, Phnom Penh, Cambodia. A total of fifteen healthy male Cambodian volunteers, aged between 18 and 30 years, weighing 51 to 65 kg participated in the study. Inclusion criteria included absence of significant abnormal findings on history or examination, particularly liver, kidney, cardiovascular diseases or peripheral neuropathy; absence of a history of antimalarial drug ingestion in the preceding three months; and absence of other drugs or medications ingested in the preceding week. None were smoker or alcohol drinkers or were on regular medications. Exclusion criteria included those who were HIV, HBsAg or HCAg positive. Written informed consent for participation was obtained from all the volunteers before initiation of the study. The study was approved by the Ethics Committees of the Ministry of Health, Cambodia.

On enrollment, a medical history was taken, including a full physical examination. Each volunteer had a thorough physical examination and routine laboratory investigations, plain chest x-ray, urinalysis and a 12-lead electrocardiogram (ECG).

Drug administration and study design

The study was a descriptive, open, pharmacokinetic investigation. Artesunate and mefloquine were given in single oral doses. Trial drugs were administered as 65 mg for the artesunate sachet (each sachet contains 65 mg artesunate with a total weight of 130 mg powder; Cambodian Pharmaceutical Enterprise) and 100 mg for the mefloquine sachet (each sachet contains 100 mg mefloquine with a total weight of 200 mg powder; Cambodian Pharmaceutical Enterprise). The artesunate was given to all subjects in a fixed, single dose of 4 mg/kg body weight, concurrently with a single dose of 10 mg/kg body weight mefloquine. All volunteers fasted overnight before drug administration. Compliance with all drug intake was under the investigators' supervision. No food was allowed until 2 hours after drug intake. The volunteers were hospitalized for 7 days. No other concurrent drugs or alcohol were taken two weeks prior to or during the study period.

Blood sample collection

Serial venous blood samples (2-5 ml each) were collected through an indwelling intravenous Teflon™ catheter, inserted into a forearm vein of the subject; the patency was maintained with sodium-heparinized saline. The sampling times were at 0 (before drug administration), 15, 30, and 45 minutes, and at 1.0, 1.5, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, 72, 96, 120, 144,

and 168 hours after drug administration. A 3-ml blood sample for the determination of the plasma concentrations of artesunate and its active metabolite dihydorartemisinin was obtained and centrifuged at 3,000 rpm for 15 minutes. The plasma samples were separated and stored at -180ºC in a liquid nitrogen tank. Two-ml blood samples were immediately stored frozen at -20ºC as whole blood samples for the measurement of mefloquine concentrations. The plasma and whole blood samples were transported for analysis at the Pharmacology of Infectious Diseases Research and Service Unit, Faculty of Allied Health Sciences, Thammasat University, Thailand, where they were stored at -80ºC until analysis.

Adverse reaction monitoring

All volunteers were physically examined and adverse reactions during the study were recorded with the date and time at which they occurred and disappeared. Adverse effects were assessed on the basis of non-suggestive questioning by the study investigators. These included gastrointestinal, central nervous system, cardiovascular, and dermatological effects, as well as other changes possibly attributable to the study drugs. Routine blood investigations (hematology and biochemistry), and urinalysis were performed prior to and at the end (day 7) of the study.

Drug analysis

The concentrations of artesunate and dihydroartemisinin were measured by high performance liquid chromatography (HPLC) with electrochemical detection, according to the method described by Na-bangchang *et al* (1998b). The limits of quantification for both artesunate and dihydroartemisinin (α-anomer) in spiked plasma samples were 5 and 3 ng/ml, respectively. The average recovery of artesunate at the concentration range of 5-1,200 ng/ml was 88.9%. The coefficients of variation for the inter-assay were below 10% for both compounds at concentrations of 50, 200 and 1,200 ng/ml, and below 20% at a concentration of 10 ng/ml.

Concentrations of mefloquine in whole blood were measured by HPLC with fluorescence detection using the method described by Karbwang *et al* (1989). The limit of quantification in the spiked whole samples was 3 ng/ml. The average recovery of mefloquine at the concentration range of 10-3,200 ng/ml was 89.3%. The coefficients of variation for the inter-assay were below 10% at the concentrations of 50, 200, 400, 800, 1,600, and 3,200 ng/ml, and below 20% at a concentration of 10 ng/ml.

Pharmacokinetic analysis

The pharmacokinetic parameters of mefloquine, artesunate, and dihydroartemisinin following the administration of the drug combination were calculated by the model-independent method (Gibaldi, 1991). The time at which the maximum plasma concentration occurred (t_{max}), and the maximum concentration (C_{max}) were obtained directly from the concentration-time data. The area under the curve from zero time to the last observed time (AUC_{0-t}) was calculated by the linear trapezoidal rule for ascending data points and by the log-trapezoidal rule for descending data points. The area under the curve extrapolated from the last data point to infinity ($AUC_{t-\infty}$) was estimated by dividing the estimated concentration at the last data point with the elimination rate constant (λ_z). The total area under the curve ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + AUC_{t-\infty}$. The terminal elimination rate constant (λ_z) and half-life ($t_{1/2z}$) were estimated by log-linear regression of at least four last concentration-time data.

Statistical analysis

Statistical analysis of the data was performed with SPSS for Windows (SPSS Software, Gorichem, Netherlands). The distribution of data was assessed for normality using the Schapiro-Wilks test; normally distributed variables were expressed as means with 95% confidence intervals (95% CIs); data which were not normally distributed were expressed as medians with 95% CIs. Comparison of the variables among the three groups was done by the Friedman two-way ANOVA test and comparison of the variables between the two groups was done by the Mann-Whitney *U* test. The significance level for all the tests was set at α <0.05.

RESULTS

Tolerability

All the volunteers were healthy, verified by

laboratory results, physical examination, and vital sign monitoring. Table 1 presents the demographic and baseline laboratory (hematology/ biochemistry) data for the volunteers. Only two subjects experienced mild (CTC grade 1) and self-limited nausea and diarrhea following drug administration. Nausea occurred within 30 minutes, while diarrhea occurred 6 hours after drug administration. These symptoms disappeared after one hour without any treatment. No serious adverse events or significant changes in any ECG parameters from baseline were observed. Significant changes were observed with some laboratory parameters on day 3 and/or day 8 after drug administration. These included a decrease in hemoglobin, hematocrit, BUN and an increase in creatinine levels. These changes had no clinical significance.

Pharmacokinetics

Median plots of concentration-time profiles of dihydroartemisinin and mefloquine following the administration of artesunate (4 mg/kg body weight) concurrently with mefloquine (10 mg/kg body weight) in 15 healthy Cambodian male volunteers are shown in Figs 1a and 1b.

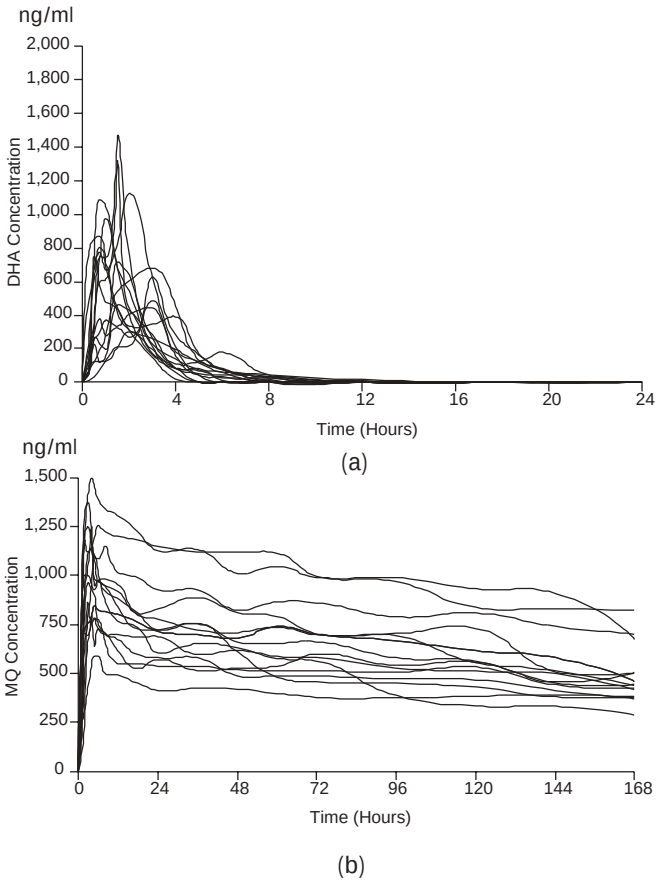

Fig 1–Concentration-time profiles of (a) dihydroartemisinin (DHA) and (b) mefloquine (MQ) following the administration of a 4 mg/kg body weight artesunate given concurrently with 10 mg/kg body weight mefloquine.

Artesunate and dihydroartemisinin. Considerable inter-individual variation in plasma concentrations of artesunate and dihydroartemisinin was observed. Approximately 50% of the volunteers (8 out of 15 subjects) had detectable concentrations of artesunate within 15 minutes of drug administration. The artesunate formulation was rapidly absorbed and extensively biotransformed to dihydroartemisinin; significant plasma concentrations of artesunate were detectable as early as 15 minutes after dosing. Concentrations of the metabolite dihydroartemisinin were detectable in the plasma of all the subjects as early as 15 minutes after the artesunate dose. The artesunate was rapidly transformed into dihydroartemisinin and thus, its concentrations were low during the investigation period (median peak concentration of 103 ng/ml at 45 minutes). In most cases, the concentrations declined to levels below the limits of quantification (LOQ: 5 ng/ml) 5-6 hours after the artesunate dose. Plasma concentrations of dihydroartemisinin on the other hand, were above the LOQ (3 ng/ml) at 8 hours after the artesunate dose in most cases.

The bioavilability/pharmacokinetic analysis of the artesunate plasma concentration-time profiles was not performed due to the low and unpredictable concentration-time profiles of this parent compound. The bioavailability/pharmacokinetics of dihydroartemisinin in the 15 healthy male Cambodian volunteers following the administration of 4 mg/kg body weight of artesunate concurrently with mefloquine (10 mg/kg body weight) was analysed by the model-independent pharmacokinetic analysis approach as summarized in Table 2. Inter-individual varia-

Table 1
Demographic and baseline laboratory data of 15 healthy male Cambodian volunteers; data are presented as median (95% CI) values.

Age (y)	20 (18-30)
Body weight (kg)	56 (51-65)
Hematology	
Hemoglobin (g/dl)	14.4 (13.2-16.4)
Hematocrit (%)	43.4 (38.8-49.2)
Platelets (x 10^9 /l)	268 (202-314)
White cells (x 10^9 /l)	7.4 (4.5-11.9)
PMN (x 10^9 /l)	4.48 (2.08-6.51)
Lymphocyte (x 10^9 /l)	2.21 (1.33-4.28)
Monocyte (x 10^9 /l)	0.24 (0.06-0.8)
Eosinophil (x 10^9 /l)	0.22 (0.09-3.57)
Biochemistry	
Direct bilirubin (mg/dl)	0.30 (0.24-0.47)
Total bilirubin (mg/dl)	0.56 (0.40-1.22)
Alkaline phosphatase (units/ml)	174 (105-361)
SGOT (units/ml)	20 (17-27)
SGPT (units/ml)	20 (14-25)
Total protein (g/dl)	8.3 (7.0-9.3)
Albumin (g/dl)	5.0 (4.4-5.4)
Creatinine (mg/dl)	0.84 (0.73-1.08)
BUN (mg/dl)	21 (16-50)
Glucose (mg/dl)	81 (71-103)

tion in all the pharmacokinetic parameters varied between 38 and 53%, with the highest variation in the t_{max}. A median (95% CI) C_{max} of 748 (304-1,470) ng/ml achieved at 1.5 (0.3-3.0) hours (t_{max}) after drug administration. The median (95% CI) values for $AUC_{0-\infty}$, λ_z and $t_{1/2z}$ were 1.673 (1.08-2.88) μg.h/ml, 0.54 (0.24-1.1)/hour and 1.3 (0.6-2.9) hours, respectively.

Mefloquine. All the subjects had detectable concentrations of mefloquine within the first hour (first blood sampling time) of drug administration. At 168 hours, the median (95% CI) whole blood concentration was 436 (286-822) ng/ml. The pharmacokinetics of mefloquine in 15 healthy male Cambodian volunteers following the administration of 10 mg/kg body weight of mefloquine concurrently with artesunate (4 mg/kg body weight) analysed by the model-independent pharmacokinetic analysis approach are summarized in Table 3. Inter-individual variation in pharmacokinetic parameters varied between 24 and 28%. A median (95% CI) C_{max} of 1,000 (591-1,500) ng/ml was observed at 4 (2-6) hours (t_{max}) after drug administration. A median (95% CI) value for AUC_{0-168h} was 3.92 (2.88-7.02) μg.h/ml.

Table 2
Pharmacokinetics of dihydroartemisinin in 15 healthy male Cambodian volunteers following the administration of 4 mg/kg body weight artesunate given concurrently with 10 mg/kg body weight mefloquine; data are presented as individual parameters and median (95% CI) values.

Patient no.	C_{max} (ng/ml)	t_{max} (h)	λ_z (/h)	$t_{1/2z}$ (h)	$AUC_{0-\infty}$ (μg.h/ml)
1	304	2	0.24	2.88	1.134
2	442	3	0.52	1.31	1.649
3	802	0.75	0.41	1.67	2.038
4	1,470	1.5	0.47	1.46	2.844
5	715	1.5	0.54	1.27	1.589
6	1,090	0.75	0.88	0.78	1.953
7	460	1.5	0.70	0.98	1.08
8	870	0.75	1.05	0.66	1.517
9	1,120	2	1.1	0.63	2.779
10	1,320	1.5	0.52	1.32	2.087
11	748	0.5	0.43	1.61	2.116
12	626	3	0.46	1.50	1.673
13	487	3	0.77	0.89	1.427
14	972	1	0.77	0.90	1.653
15	680	3	0.66	1.04	2.376
Median	748	1.5	0.54	1.30	1.673
(95%CI)	(304-470)	(0.5-3)	(0.24-1.10)	(0.6-2.9)	(1.08-2.844)

Table 3
Pharmacokinetics of mefloquine in 15 healthy male Cambodian volunteers following the administration of 10 mg/kg body weight of mefloquine given concurrently with 4 mg/kg body weight artesunate; data are presented as individual parameters and median (95% CI) values.

Patient No.	C_{max} (ng/ml)	t_{max} (h)	AUC $_{0-168h}$ (μg.h/ml)
1	862	3	3.76
2	591	5	2.8
3	1150	2	4.84
4	1250	3	3.76
5	781	5	3.56
6	1000	3	3.48
7	961	3	3.51
8	796	4	3.92
9	848	5	4.59
10	885	3	3.82
11	1500	4	7.02
12	1260	6	6.87
13	1370	3	5.87
14	1250	4	4.86
15	1100	5	4.33
Median	1,000	4	3.92
(95% CI)	(591-1,500)	(2-6)	(2.80-7.02)

DISCUSSION

The vicious emergence of multidrug resistant *Plasmodium falciparum* poses a major problem in several tropical regions, especially Southeast Asia (Berman *et al,* 2001). The 4-quinolinemethanol antimalarial mefloquine is currently the mainstay for prophylaxis and treatment of uncomplicated falciparum malaria. It has proved to be relatively well tolerated and effective against all malaria species in man including multidrug resistant falciparum malaria, either when given alone or in combination with artemisinin derivatives (artesunate, artemether, and dihydroartemisinin). The combination of mefloquine with artesunate is commonly used in areas with marked mefloquine resistance. Successful therapy with this combination is, therefore, dependent on the pharmacokinetic and bioavailability characteristics of the drug. In the absence of a parenteral formulation, reliable and adequate drug absorption (rate and extent) after oral administration needs to be ensured in order to achieve prompt and adequate systemic exposure to the drugs. Several commercial tablet formulations of artesunate and mefloquine are now being marketed worldwide. There is evidence of a lack of bioequivalence for the generic forms of both these drugs (Na-Bangchang *et al*, 1998a, 2000). In the present study, we have investigated the bioavailability/pharmacokinetics of new pediatric formulations of artesunate and mefloquine manufactured by Cambodian Pharmaceutical Enterprise, when given concurrently as single oral doses. These two formulations are co-packaged, and it is the intention that in Cambodia, they should never be used singly, the bioavailability/pharmacokinetics of artesunate/dihydroartemisinin and mefloquine were, therefore, investigated following the co-administration of both drugs. The bioavailability/pharmacokinetics of mefloquine is well documented. Full analysis of blood concentration-time profiles of mefloquine until the terminal phase of elimination (a minimum of 42 days) was considered unnecessary. The study was a descriptive, open study design, and thus the standard study design for bioequivalence which involves the randomized, cross-over design used by the Food and Drug Administration (FDA, 1992) was not applicable to this present study. A description of the bioavailability/pharmacokinetics of mefloquine focused only on the first seven days after drug administration, since it is during this period that adequate plasma/blood concentrations of antimalarial drugs can ensure a radical cure. The artesunate/mefloquine combination was well tolerated. No severe and/or serious adverse effects were seen in any of the subjects. Only two subjects experienced mild and self-limited nausea and diarrhea following drug administration. These adverse effects are well-documented for mefloquine, but not artesunate (Karbwang *et al*, 1988, 1998; Na-Bangchang *et al*, 1991, 1995, 1999, 2000).

Bioavailability/pharmacokinetics of artesunate and dihydroartemisinin

The pharmacokinetics of artesunate and dihydroartemisinin when given concurrently at single oral doses of 4 and 10 mg/kg body weight,

which is in accordance with other reports (Na-Bangchang *et al*, 1991, 1998a). The artesunate was poorly absorbed from the gastrointestinal tract but was detectable in the plasma as early as 15 minutes after ingestion, but at very low levels during 0-6 hours (in most cases). The low systemic availability of artesunate can be explained in part by poor gastrointestinal absorption, and also to its extensive metabolism by hepatic and mucosal enzymes, mainly to its active plasma metabolite dihydroartemisinin, and, to a lesser extent, other metabolites. Marked inter-subject variation in plasma levels of artesunate and thus dihydroartemisinin can be attributed to inter-individual variability, the functional integrity of the upper gastrointestinal tract, gastric pH and metabolic capacity.

The very short systemic exposure of artesunate compared with other artemisinin derivatives (Na-Bangchang *et al*, 1991, 1998a) can be explained by the relatively high polarity characteristics of the drug molecule. Despite the observation of the rapid decline of artesunate, significant detectable plasma concentrations during this time period (0-6 hours) were noted. This is in contrast to a previous study where almost undetectably low levels of artesunate were reported after dosing (Bernakis *et al*, 1997). Our observations raise the argument over the view on the pro-drug characteristic of artesunate (immediate and complete hydrolysis to the active metabolite before entering the systemic circulation). The bioavailability/pharmacokinetics of dihydroartemisinin were governed by the kinetic characteristics of the parent drug; the formation of this metabolite was rate-limited by the kinetics (absorption, distribution, biotransformation, excretion) of artesunate.

With regard to oral bioavailability, the results show that the artesunate formulation manufactured by Cambodian Pharmaceutical Enterprise was bioavailable to an even greater extent than the other two widely used pharmaceutical formulations reported previously by our group (Artesunate-Gulillin™, China and Artesunate-Arenco™, Belgium) (Na-Bangchang *et al,* 1998b, 2000). When expressed as mg per kg of body weight, the bioavailability parameters of dihydroartemisinin representing the extent of absorption (C_{max} and $AUC_{0-\infty}$) were significantly greater than that observed with the other two formulations (although statistical significant difference was observed only with the C_{max}). The C_{max} was, 2.4 and 5.0 times as high,

Table 4
Pharmacokinetics of dihydroartemisinin following the administration of the three formulations of artesunate (Artesunate-Cambodia™, Artesunate-Guilllin™, and Artesunate-Arenco™). Data are presented as median (95%CI) values.

Pharmacokinetic parameters	Artesunate-Cambodia™ (n=15)	Artesunate-Guilllin™ * (n=10)	Artesunate-Arenco™ * (n=10)
Dose	4 mg/kg body weight	300 mg (10-15mg/kg body weight)	300 mg (10-15 mg/kg body weight)
C_{max} (ng/ml/mg/kg body weight)	0.0576 (0.0226-0.1269)[a]	0.0245 (0.0164-0.0431)	0.0114 (0.0054-0.0182)
t_{max} (h)	1.5 (0.5-3.0)	1.25 (0.25-2.5)	1.25 (0.5-3.0)
$AUC_{0-\infty}$ (ng.h/ml/mg/kg body weight)	0.147 (0.084-0.267)	0.128 (0.098-0.213)	0.131 (0.036-0.218)
$t_{1/2z}$ (h)	1.3 (0.6-2.9)[b]	3.8 (1.7-6.4)	1.6 (1.3-2.9)

[a] = Statistically significant difference between Artesunate-Cambodia formulation and Artesunate-Guillin formulation (p=0.0008. 95%CI -0.00611 to -0.017) and Artesunate-Arenco formulation (p=0.0019; 95%CI -0.0719 to -0.0230).

[b] = Statistically significant difference between Artesunate-Cambodia formulation and Artesunate-Guillin formulation (p=0.0002. 95%CI 1.36 to 3.69) and Artesunate-Arenco formulation (p=0.0107; 95%CI 0.17 to 1.17)

* Data obtained from Na-Bangchang *et al*, 1998a

Table 5
Pharmacokinetics of mefloquine following the administration of the two formulations of mefloquine (Mefloquine-Cambodia™ and Lariam™; Roche). Data are presented as median (95%CI) values.

Pharmacokinetic parameters	Mefloquine-Cambodia™ (n=15)	Lariam™ (n=10)
Dose	10 mg/kg body weight	750 mg (10-15 mg/kg body weight)
C_{max} (ng/ml/mg/kg body weight)	0.0331 (0.0203-0.0461)[a]	0.0228 (0.01754-0.03172)
t_{max} (h)	4 (2-6)[b]	6 (3-12)
AUC_{0-168h} (μg.day/ml/mg/kg body weight)	0.00014 (0.000093-0.00022)[c]	0.000096 (0.0000842-0.000143)

[a] = Statistically significant difference between Mefloquine-Cambodia formulation and Lariam® formulation (p=0.0112; 95%CI -0.0132 to -0.0022).
[b] = Statistically significant difference between Mefloquine-Cambodia formulation and Lariam® formulation (p=0.032; 95%CI 0 to 3).
[c] = Statistically significant difference between Mefloquine-Cambodia formulation and Lariam® formulation (p=0.010; 95%CI -0.000063 to -0.000008).
* Data obtained from Karbwang *et al*, 1991d.

respectively, as that following the administration of the artesunate formulations manufactured by Guillin and Arenco n.v. Pharmaceuticals (Table 4). The rate of absorption reflected by t_{max} was comparable with that observed with the other two formulations. The higher $AUC_{0-\infty}$ of dihydroartemisinin was observed despite the fact that $t_{1/2z}$ was shorter than the other two formulations (0.3 and 0.8 times) (Table 4).

Bioavailability/pharmacokinetics of mefloquine.

The rate of mefloquine absorption from the gastrointestinal tract, and particularly the rate of drug elimination, was relatively slow compared with dihydroartemisinin. The absorption kinetics of mefloquine when given as a single oral dose of 10 mg/kg body weight concomitantly with a single oral dose of 4 mg/kg body weight artesunate was in general, in accord with the previously reported studies (Karbwang *et al*, 1998; Na-Bangchang *et al*, 1995, 1999). Mefloquine is currently available on the market in at least three formulations, *ie,* Lariam™ (Roche, Basel, Switzerland), Mephaquin 100 Lactab™ (Mepha Pharmaceuticals, Switzerland) and Eloquine-250™ (Moltar). Lariam™ is the formulation which is widely used and registered in several countries worldwide. It has been reported by our group that the three formulations are not bioequivalent (Na-Bangchang *et al*, 2000). Results from the present study, however, show that the mefloquine formulation manufactured by Cambodian Pharmaceutical Enterprise was bioavailable to an even greater extent than the other two widely used pharmaceutical formulations previously reported by our group (Karbwang *et al*, 1992). When expressed as mg per kg body weight, the bioavailability parameters of mefloquine representing extent (C_{max} and AUC_{0-168h}) and rate of absorption (t_{max}) were significantly greater than those observed with Lariam™ (although there was statistical significant only with the C_{max}). The C_{max} and AUC_{0-168h} were 1.5 times as high as those following Lariam™ (Table 5).

In view of clinical efficacy and toxicity, the observed satisfactory bioavailability of the artesunate/mefloquine formulations produced by the Cambodian Pharmaceutical Enterprise could have a significant impact on treatment and prophylactic outcomes, as well as on the tolerability profiles of both drugs, particularly if these effects are concentration-dependent. Threshold mefloquine levels for therapeutic and prophylactic efficacy and tolerability have not been clearly defined. It has been shown that whole blood mefloquine concentrations/ bioavailability during the first two days of treatment are important determinants of treatment outcome. Patients with treatment failure have significantly lower mefloquine concentrations

than those with successful treatment outcomes (Karbwang *et al*, 1991b; 1993). Concentrations of less than 400 ng/ml have been shown to increase the risk of prophylaxis failure (Lobel *et al,* 1993), and concentrations below 1,000 ng/ml have been associated with a 2.5-fold increased risk of treatment failure in patients with uncomplicated falciparum malaria along the Thai-Myanmar border (ter Kuile *et al*, 1992).

The good bioavailability profile of the artesunate/mefloquine formulation produced in Cambodia observed in this study suggests that both formulations may be used as alternatives to the currently available formulations for the treatment and prophylaxis of falciparum malaria with a satisfactory safety profile.

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