PHARMACOKINETICS OF THE FOUR COMBINATION REGIMENS OF DIHYDROARTEMISININ/MEFLOQUINE IN ACUTE UNCOMPLICATED FALCIPARUM MALARIA

Kesara Na-Bangchang[1], A Thanavibul[1], P Tippawangkosol[2] and J Karbwang[1]*

[1]Clinical Pharmacology Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok; [2]Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Abstract. The pharmacokinetics of oral dihydroartemisinin and mefloquine were investigated in 40 patients (aged 16-30 y, weighing 45-60 kg) with acute uncomplicated falciparum malaria following the four combination regimens of dihydroartemisinin/ mefloquine [regimen-I: 300 mg dihydroartemisinin (h-0) plus 750 mg mefloquine (h-0); regimen-II: 300 mg dihydroartemisinin (h-0) plus 750 mg mefloquine (h-24); regimen-III: 300 mg dihydroartemisinin (h-0) plus 750 and 500 mg mefloquine (h-24 and 30); regimen-IV: 300 mg dihydroartemisinin (h-0) plus 750 and 500 mg mefloquine (h-0, 24)]. The four combination regimens were well tolerated. Patients in all treatment groups had a rapid initial response. However, 9 patients (4, 4, and 1 cases in regimens-I, II, and IV) had reappearance of parasitemia during the follow-up period. Significant changes in the pharmacokinetic parameters of both mefloquine and dihydroartemisinin were observed in patients with malaria compared with healthy subjects reported in a paralleled study. For mefloquine, C_{max} (mg per dose), $AUC_{0\text{-}day1}$ (mg per dose), and $AUC_{0\text{-}day7}$ (mg per dose) were significantly higher in patients. Furthermore, $t_{max,}$ was prolonged while V_z/F contracted and $t_{1/2,z}$, MRT shortened in patients with malaria. For dihydroartemisinin, C_{max}, AUC, t_{max} and V_z/F were changed in the same direction as mefloquine, whereas $t_{1/2z}$ and MRT were prolonged. CL/F was also significantly reduced in patients with malaria. Absorption/disposition kinetics of oral dihydroartemisinin were similar among the various regimens. On the other hand, $AUC_{0\text{-}day1}$ (mg per dose) of mefloquine after regimen-III was significantly higher than the other three regimens. Combination regimens with two divided doses of mefloquine (regimens-III and IV) resulted in a significantly delayed t_{max} (especially regimens-IV) compared with those with single dose regimens (regimens-I and II).

INTRODUCTION

Malaria remains a major health problem worldwide due to the emergence of multidrug resistant *Plasmodium falciparum* (Wernsdorfer, 1994). In the face of this ominous situation, artemisinin and its derivatives (artemether, artesunate, arteether, dihydroartemisinin) have lately become a renewed hope for combating the emerging generations of resistant malaria (Myint *et al*, 1989; Harinasuta and Karbwang, 1994; Hein, 1994; Li *et al,* 1994). These drugs have gained considerable prominence in the chemotherapy of both uncomplicated and severe falciparum malaria by the demonstrated high activity against multidrug resistant falciparum strains with low toxicity profiles. Due to the high recrudescence rates from monotherapeutic regimens of artemisinin derivatives (Harinasuta and Karbwang, 1994), however, the use of these drugs in combination with drugs with long half-lives, such as mefloquine, has been increasingly advocated for achievement of radical cure. There are potential advantages of combining artemisinin derivatives with mefloquine in reducing the dose and treatment period of the first, which will improve compliance in clinical practice. Short course combination (2-days) regimens of artemether or artesunate with mefloquine have proved effective with good patient

Present address for 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

*Present address: Product Research and Development, TDR/WHO, Geneva, Switzerland

compliance (Bunnag *et al,* 1996; Na-Bangchang *et al,* 1997).

Dihydroartemisinin is an active metabolite of artemether, artesunate, and arteether (Lee *et al,* 1990). It is currently in clinical use as formulated tablets/capsules and suppositories. In addition to artemether or artesunate, the use of dihydroartemisinin in combination with mefloquine may also render another promising combination partner for the treatment of multidrug resistant falciparum malaria. In a previous investigation, we demonstrated the lack of adverse pharmacokinetic drug interactions, safety, and synergistic antimalarial activity (*ex vivo* blood schizontocidal activity) of this combination in healthy Thai subjects (Na-Bangchang *et al,* 1999a). To our knowledge, there has been no information on the pharmacokinetics of dihydroartemisinin and mefloquine when used in combination in patients with malaria. This pharmacokinetic study was carried out as a part of a comparative clinical trial (Na-Bangchang *et al,* 1999b) for the assessment of clinical efficacy and tolerability of four regimens of dihydroartemisinin/mefloquine in multidrug resistant falciparum malaria.

METHODS

Patients

Forty patients with acute uncomplicated falciparum malaria (asexual form parasitemia less than 5% with symptoms, *eg* fever), aged between 16 and 30 years, weighing 45 to 60 kg, with no history of liver or kidney diseases or a history of previous antimalarial treatment within 1 month were recruited into the study (confirmed by the measurement of plasma levels of mefloquine, quinine, artemether, artesunate, and dihydroartemisinin) (Karbwang *et al*, 1987, 1989a,b; Na-Bangchang *et al,* 1988). Written informed consent for participation was obtained from all patients before initiation of the study. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

On admission, each patient had a thorough physical examination and routine laboratory investigations, plain chest x-ray, urinalysis and a 12-lead electrocardiogram (ECG). The patients were admitted to the Bangkok Hospital for Tropical Diseases for 42 days.

Treatment

Study participants were allocated at random to receive four combination regimens of dihydroartemisinin/mefloquine as follows:

Regimen-I. a single oral dose of 300 mg dihydroartemisinin (50 mg per capsule, Arenco n.v., Belgium), given concurrently with a single oral dose of 750 mg mefloquine (Lariam, Roche®, 250 mg per tablet);

Regimen-II. an initial single oral dose of 300 mg dihydroartemisinin, followed by a single oral dose of 750 mg mefloquine after 24 hours;

Regimen-III. an initial single oral dose of 300 mg dihydroartemisinin, followed by two oral doses of 750 and 500 mg mefloquine given at 24 and 30 hours after dihydroartemisinin; and

Regimen-IV. a single oral dose of 300 mg dihydroartemisinin given concurrently with a single oral dose of 750 mg, then followed by a single oral dose of 500 mg mefloquine after 24 hours.

Compliance with all drug intake was under the investigators' supervision. No food was allowed until 3 hours after drug intake. Concomitant treatment with drugs other than paracetamol and chlorphenhydrinate were avoided during the first week of the investigation period. Patients who had treatment failures from any regimen were retreated with a 5-day regimen of oral artemether (300 mg initially, then 100 mg daily for another 4 days).

Patients who developed *P. vivax* infection during the follow-up period were given 150 mg (base) of chloroquine to suppress the symptoms; a full course was given on discharge.

Clinical and laboratory assessments

Following drug intake, parasite counts were performed every 6 hours until negative, then once daily until day-7, and once weekly until day-42. Peripheral blood smears were taken for malaria parasite identification; parasites in thick and thin smears were identified by Giemsa stain, and parasite counts were reported per 1,000 RBCs or per 200 WBCs.

The patients were physically examined and

adverse reactions during the study were recorded with the date and time at which they occurred and disappeared. These changes included gastrointestinal, central nervous system, cardiovascular, dermatological and hematological effects, as well as other changes possibly attributable to dihydroartemisinin and mefloquine. Adverse effects were assessed on the basis of non-suggestive questioning by the study investigators. Heart rate, blood pressure, and ECGs were recorded at intervals during frequent blood sampling for pharmacokinetic evaluation, then daily until day-7 and weekly until day 42. Hematological examinations and biochemical screens were performed on days 0, 2, 4, 7 then weekly until day 42. These included complete blood counts, liver and kidney function tests and measurements of electrolyte levels.

Only those patients who completed the 42 day follow-up period were included in the efficacy analysis. Clinical evaluation was based on the parasite clearance time (PCT: the time taken for the parasite count to fall below the level of microscopic detection), fever clearance time (FCT: the time taken for the temperature to drop below 37.3ºC, and remain at that value for at least 24 hours), cure rate, frequency and severity of adverse clinical and laboratory reactions. The time to reach 50 and 90% of the original parasite count (PCT_{50} and PCT_{90}) were determined by simple linear interpolation of the parasite count-time data.

Blood collection for pharmacokinetic study

Venous blood was collected for determination of dihydroartemisinin and mefloquine levels, through an antecubital catheter, into heparinized plastic tubes.

Dihydroartemisinin assay: serial blood samples (5 ml each) were collected immediately before, and at 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 18, 24, 30, 36, and 48 hours after drug administration. Plasma samples were obtained by centrifugation at 2,000*g* within 10 minutes and stored at -80ºC until analysis.

Mefloquine assay: three ml of blood was collected prior to and after drug administration (on day-0 or day-1), at hours 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, and on days 3, 4, 5, 6, 7, 14, 21, 28, 35 and 42. Whole blood samples were stored at -80ºC until analysis.

Drug analysis

Concentrations of dihydroartemisinin (α-anomer) and mefloquine in plasma and whole blood, respectively, were measured by high performance liquid chromatography according to the methods of Na-Bangchang *et al* (1998) and Karbwang *et al* (1989). The average recoveries of dihydroartemisinin and mefloquine at the concentration ranges of 10-1,200 and 10-6,500 ng/ml were 88.2-100% and 79-92%, with inter- and intra-assay coefficients of variation below 20% at 10 ng/ml and below 10% at concentrations above this value. The stability of dihydroartemisinin (100 and 500 ng/ml) in plasma has been observed for up to 1 year and found to be within ±10% of replicate samples stored at -80ºC.

Pharmacokinetic analysis

The pharmacokinetic parameters of dihydroartemisinin and mefloquine were calculated by the model-independent method from the plasma or whole blood concentration-time data (Gibaldi, 1991). The time at which maximum concentration occurred (t_{max}), and the maximum concentration (C_{max}) were obtained directly from concentration-time data. The area under the curve from zero time to the last observed time (AUC_{0-t}) or certain specific time points were 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 extrapolations contributed, on average, 1.5 and 7.2% for dihydroartemisinin and mefloquine, respectively. The total area under the curve (AUC) 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. The apparent total body clearance (Cl/F) and apparent volume of distribution associated with the terminal phase (V_z/F) were calculated as Cl/F=dose/AUC and $V_z/F = (Cl/F)/\lambda_z$, respectively. The area under the statistical moments curve

(AUMC) was calculated by multiplication of the concentration and time after dosage for all observations. The mean residence time (MRT) was calculated as AUMC/AUC.

To better characterize the absorption phase, a one- or two-compartment open model with first order input and first-order elimination was fitted to the data by an iterative least squares curve fitting program TopFit®. The observed concentrations were weighted as the reciprocal of the analytical variance. The adequacy of the pharmacokinetic models chosen was based on statistical methods to assess the validity of the models for describing the experimental data *ie,* F-ratio test, Akaike's information, Schwartz and Imbimbo criteria.

Statistical analysis

Statistical analysis of the data was performed with SPSS for Windows (SPSS Software, Gorichom, The 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% CI); data which were not normally distributed were expressed as medians with 95% CI.

The initial parasitemia, demographic data, PCT, PCT_{50}, PCT_{90} and FCT and pharmacokinetics of dihydroartemisinin and mefloquine in the patients following the administration of all combination regimens were compared using the one-way ANOVA and modified *t*-test (Bodferronni test) for normally distributed data, or by the Kruskal Wallice test and Mann-Whitney *U* test for non-normally distributed data. Statistical significance for both tests was set at $p \leq 0.05$.

RESULTS

Clinical response

Patients with acute uncomplicated falci-

Table 1
Demographic and laboratory data of 40 patients with uncomplicated falciparum malaria following the four regimens of dihydroartemisinin/mefloquine; data are presented as median (95% CI) values.

	Regimen-I	Regimen-II	Regimen-III	Regimen-IV
Age (y)	21 (19-22)	21 (17-25)	25 (19-32)	19.5 (19-30)
Body weight (kg)	53.8 (49-58)	53.8 (45-60)	51.5 (45-59.8)	51.7 (45-57)
Body temperature (ºC)	38.0 (37.4-38.5)	37.9 (36.8-39.2)	37.6 (36.5-39.7)	38.1 (37.3-40.3)
*Initial parasitemia (/μl)	15,075 (10,500-25,130)	14,660 (8,700-80,100)	14,932 (109,710)	14,825 (5,180-136,170)
Heart rate (/min)	84.5 (55-102)	74 (54-88)	78 (55-88)	83 (60-104)
Systolic BP (mmHg)	105 (82-116)	103 (80-108)	111 (100-120)	111 (100-120)
Diastolic BP (mmHg)	65 (58-76)	63 (59-77)	70 (60-78)	69 (59-74)
Hemoglobin (mg/dl)	13.1 (8.3-16)	11.5 (9.5-13.6)	12.2 (9.2-13)	13.1 (7.7-12.9)
Hematocrit (%)	39.5 (28-48)	35.5 (25-46)	36.5 (28-40)	39.5 (25-42)
Red cells (x 10^6 /μl)	4.8 (3.48-5.79)	4.82 (3.56-5.34)	4.1 (3.2-4.77)	4.8 (2.65-5.18)
White cells (x 10^3 /μl)	5.0 (3.2-8.2)	7.3 (5.0-8.4)	5.9 (4.1-7.5)	5.0 (4.0-8.5)
Platelets (x 10^5 /μl)	64.0 (49-214)	123 (34-175)	94 (31-158)	64 (61-231)
Direct bilirubin (mg/dl)	0.48 (0.15-0.77)	0.25 (0.12-0.9)	0.45 (0.12-2.3)	0.3 (0.08-1.7)
Total bilirubin (mg/dl)	1.92 (0.94-2.83)	1.18 (0.56-2.83)	1.91 (0.18-4.7)	1.22 (0.8-3.6)
Alkaline phosphatase (units/ ml)	32.9 (20.7-50.1)	34.3 (29.5-52.6)	29.6 (23.8-48.2)	29.0 (22-73.4)
SGOT (units/ml)	43.5 (29-57)	40 (25-48)	28.0 (20-92)	29.0 (20-82)
SGPT (units/ml)	40 (16-52)	37.5 (16-55)	24.5 (19-40)	20.0 (15-78)
Albumin (g/dl)	4.1 (3.4-4.5)	4.0 (3.4-45)	3.9 (3.1-4.7)	4.2 (3.4-4.5)
Globulin (g/dl)	2.9 (2.9-3.1)	2.9 (2.6-3.3)	2.55 (2.4-2.9)	3.0 (2.3-3.1)
Creatinine (mg/dl)	1.15 (0.9-1.3)	1.0 (0.9-1.3)	1.0 (0.75-1.15)	0.9 (0.75-1.06)
BUN (mg/dl)	19.6 (10-24)	16.9 (8.8-24)	15.5 (8-25.2)	14.6 (8.2-26.5)

*Geometric mean (range)

Table 2
Clinical response of 40 patients with uncomplicated falciparum malaria after treatment with one of the four combination regimens of dihydroartemisinin/mefloquine; data are presented as median (95% CI) values.

	Regimen-I (n=10)	Regimen-II (n=10)	Regimen-III (n=10)	Regimen-IV (n=10)
FCT (h)	26 (14-54)	28 (12-52)	21.5 (13-83)	35.5 (14-41)
PCT_{50} (h)	7 (2.5-12)	7.3 (6-11)	10 (5.5-12)	9.8 (6-12)
PCT_{90} (h)	16.5 (12-22)	16.8 (13.5-21)	17.5 (14.5-20)	15.8 (14.5-20)
PCT (h)	31 (28-43)	36 (26-42)	33.5 (27-45)	35.5 (30-48)
S (N)	6	6	10	9
RI (N)	4 (d18,21,28,35)	4 (d13,16,19,27)	0	1 (d27)
P. vivax (N)	1 (d28)	1 (d28)	1 (d42)	1 (d49)

S = clearance of asexual parasitemia within 7 days of initiation of treatment without subsequent recrudescence
RI = clearance of asexual parasitemia in peripheral blood with regard to sensitivity, followed by recrudescence within the follow-up period

parum malaria recruited for the study showed comparable baseline characteristics (Table 1). No patients had detectable levels of mefloquine, quinine, artemether, artesunate and dihydroartemisinin on admission. All of them had mild abnormal liver function tests on admission; however, these were not significantly different between groups. The abnormal laboratory values during acute malaria infection (liver function and hematological profiles) normalized within the first 2 weeks of treatment.

Patients in all treatment groups had a rapid initial response with PCT and FCT in the ranges of 22-60, and 4-97 hours, respectively. However, 9 cases (4, 4, and 1 in regimens-I, II, and IV) had a reappearance of parasitemia between days 13 and 35. Four patients developed *P.vivax* malaria during days 28-42 (Table 2).

The four combination regimens of dihydroartemisinin/mefloquine were well tolerated. The symptoms observed after drug administration included headache, dizziness, nausea, abdominal pain and diarrhea. They were mild and self-limited. These symptoms were likely due to mefloquine. The incidence of these symptoms, particularly nausea and vomiting, appeared to be lowest in the regimens with low dose mefloquine (750 mg) given on the second day (regimen-II). No drug-related changes in the vital signs or ECG tracings were found in any patients.

Pharmacokinetics

The fitting of the plasma concentration-time curves for dihydroartemisinin and mefloquine, to a respective one- and two-compartment model with first order absorption yielded satisfactory results in all patients. Figs 1a,b depict median plasma and whole blood concentration-time profiles for dihydroartemisinin and mefloquine in patients following the administration of the four combination regimens and Table 3 and 4 summarize their pharmacokinetic parameters. Large interindividual variation among the pharmacokinetic parameters were found for both dihydroartemisinin and mefloquine, particularly with AUC and CL/F, reflected by the values of coefficients of variation for both parameters (25-55%).

Oral dihydroartemisinin was rapidly absorbed; the drug was detectable in the plasma within 15 minutes of dosing. Its elimination was essentially complete within 6-12 hours of intake. Mefloquine absorption and disposition were slower processes. C_{max} of 1,202-3,249 ng/ml were attained at 10-48 hours, and on the last day of blood sampling (day-42), most patients still had measurable concentrations of mefloquine in their blood (median concentration = 25 ng/ml).

Significant changes in the pharmacokinetic parameters of both mefloquine and dihydroartemisinin were observed in patients with malaria

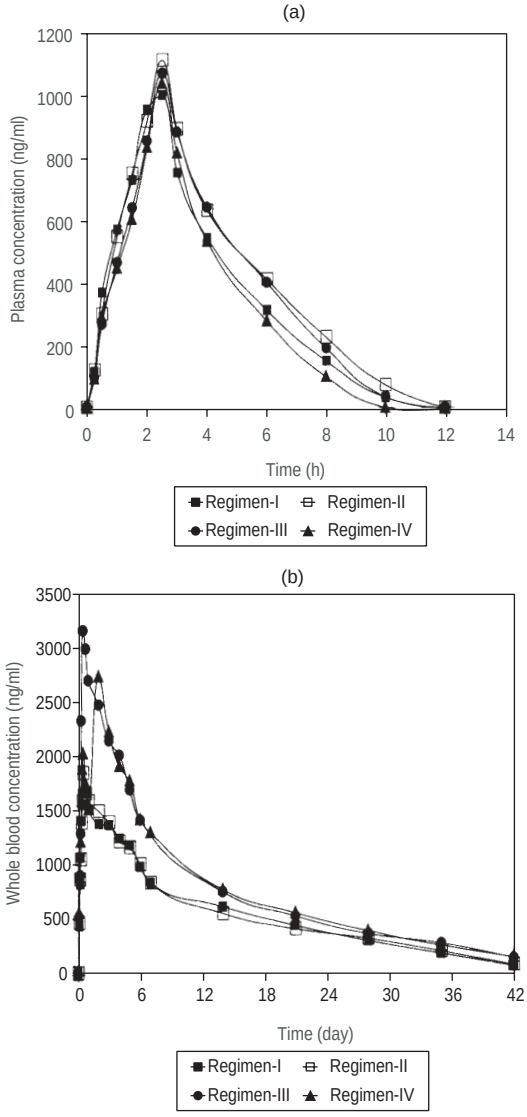

Fig 1–Median concentration-time profiles of (a) dihydroartemisinin (plasma) and (b) mefloquine (whole blood) following the four combination regimens of dihydroartemisinin/mefloquine.

compared with healthy subjects in a controlled, paralleled study in the same population (Na-Bangchang *et al*, 1999b). C_{max} and AUC of mefloquine when normalised with dose [C_{max} (mg per dose), $AUC_{0\text{-}day1}$ (mg per dose), $AUC_{0\text{-}day7}$ (mg per dose)] were significantly higher in patients. Furthermore, the t_{max} was prolonged, while the V_z/F contracted and the $t_{1/2,z}$, MRT was shortened in patients with malaria (Table 3). For dihydroartemisinin, the C_{max}, AUC, t_{max} and

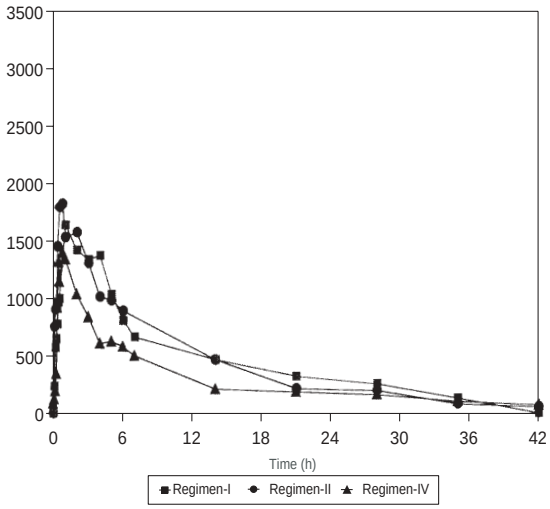

Fig 2–Median whole blood concentration-time profiles of mefloquine in 3 patients who vomited within 1 hour of mefloquine administration.

V_z/F were changed in the same direction as mefloquine, whereas the $t_{1/2,z}$ and MRT were prolonged. The CL/F was also significantly reduced in patients with malaria (Table 4).

The absorption and disposition kinetics of oral dihydroartemisinin were similar among various regimens. However, $AUC_{0\text{-}day1}$ (mg per dose) of mefloquine after regimens-III was significantly higher than the other three regimens. Combination regimens with two divided doses of mefloquine (regimen-III and IV) resulted in a significantly delayed t_{max} (especially regimen-IV) compared with those with single dose regimens (regimens-I and II).

The pharmacokinetics of dihydroartemisinin and mefloquine in patients who had a sensitive response or those with subsequent treatment failure (regimen-I, III, IV) were similar. In the 3 patients who vomited within an hour of taking the mefloquine dose (1 each in regimens-I, III, IV), all had relatively low whole blood mefloquine levels (Fig 2).

DISCUSSION

The changes in the pharmacokinetics of mefloquine during malaria infection observed in this study are consistent with several previous findings (Karbwang *et al*, 1988; Boudreau

Table 3
Pharmacokinetics of dihydroartemisinin in patients with uncomplicated falciparum malaria and healthy subjects*; data are presented as mean [95% CI] or median (95% CI) values.

Pharmacokinetic parameters	Healthy*	Regimen-I	Regimen-II	Regimen-III	Regimen-IV
C_{max} (ng/ml)	711 [569-853][a]	1,111 [1,026-1,197]	1,079 [996-1,162]	1,108 [1,027-1,188]	636 [587-819]
t_{max} (h)	1.5 (1.5-2)[b]	2.5 (2-2.5)	2.5 (2-2.5)	2.5 (2-2.5)	2.5 (2.5-2.5)
AUC (ng.h/ml)	1,811 [1,385-2,236][c]	4,463 [3,765-5,161]	4,685 [3,982-5,386]	4,332 [3,639-5,024]	4,241 [3,747-4,735]
t_{lag} (h)	0.19 (0.16-0.22)	0.18 (0.13-0.24)	0.19 (0.121-0.24)	0.20 (0.19-0.25)	0.22 (0.15-0.25)
$t_{1/2,a}$ (h)	0.62 (0.59-0.79)[d]	1.27 (1.03-1.47)	1.38 (1.08-1.53)	1.47 (1.0-1.6)	1.38 (1.26-1.57)
$t_{1/2,z}$ (h)	1.1 (0.8-1.6)[e]	1.6 (1.3-2.2)	1.9 (1.6-2.3)	1.8 (1.4-2.2)	1.9 (1.6-2.1)
MRT (h)	2.3 [1.9-2.7][f]	3.8 [3.4-4.3]	4.0 [3.6-4.4]	3.9 [3.6-4.3]	3.9 [3.6-4.1]
V_z/F (l/kg)	5.17 [4.38-5.96][g]	3.10 [2.82-3.38]	3.4 [2.98-3.82]	3.67 [3.16-4.19]	3.78 [3.36-4.21]
CL/F (ml/min/kg)	44.4 (37-85.3)[h]	23.2 (16.2-26.6)	18.2 (16.5-32.9)	22.1 (16.9-33.0)	24.8 (19.4-28.2)

*Na-Bangchang *et al,* 1999a

[a] = significantly different from regimen-I (p<0.000001, 95% CI -578, -223), regimen-II (p<0.000001, 95% CI -592, -238), regimen-III (p<0.000005, 95% CI -544, 192), and regimen-IV (p=0.000001, 95% CI -571, -222);

[b] = significantly different from regimen-I (p=0.00042, 95% CI -1.0, -0.5), regimen-II (p=0.00026, 95% CI -1.0, -0.5), regimen-III (p=0.00015, 95% CI -1.0, -0.5), and regimen-IV (p=0.000077, 95% CI -1.0, -0.5);

[c] = significantly different from regimen-I (p< 0.000001, 95% CI -3529), regimen-II (p<0.000001, 95% CI -3753, -1993), regimen-III (p<0.000001, 95% CI -3392, -1649), and regimen-IV (p< 0.000001, 95% CI -3129, -1731);

[d] = significantly different from regimen-I (p=0.00065, 95% CI -0.74, -0.31), regimen-II (p=0.00037 95% CI -0.86, -0.47), regimen-III (p=0.00028, 95% CI -0.93, -0.44), and regimen-IV (p=0.00021, 95% CI -0.91, -0.6);

[e] = significantly different from regimen-I (p=0.0311, 95% CI -0.86, -0.08), regimen-II (p=0.0081 95% CI -1.14, -0.31), regimen-III (p=0.01 95% CI -1.008 -0.22), and regimen-IV (p=0.0065, 95% CI -1.1, -0.35);

[f] = significantly different from regimen-I (p<0.000001, 95% CI -2.15, -0.86), regimen-II (p=0.000001 95% CI -2.31, -1.03), regimen-III (p< 0.000001 95% CI -2.19, -1.0), and regimen-IV (p<0.000001, 95% CI -2.08, -1.04);

[g] = significantly different from regimen-I (p<0.000001, 95% CI 1.17, 2.97), regimen-II (p=0.00002 95% CI 0.82, 2.73), regimen-III (p=0.00021, 95% CI 0.49, 2.51), and regimen-IV (p=0.00053, 95% CI 0.43, 2.34);

[h] = significantly different from regimen-I (p=0.00016, 95% CI 15.76, 56.28), regimen-II (p=0.00021 95% CI 17.42, 57.6), regimen-III (p=0.00029, 95% CI 14.35, 55.23), and regimen-IV (p=0.00016, 95% CI 14.18, 54.15)

et al, 1990). The disposition, rather than the absorption kinetics of mefloquine, is likely to be influenced by malaria infection. The delayed time to maximum concentration together with the improved systemic exposure during the first 7 days could be a consequence of the contraction of apparent volume of distribution resulting from the interruption of enterohepatic recycling of mefloquine in malaria (Karbwang *et al*, 1991). This improved systemic bioavialability during the acute phase malaria would be of benefit to malaria therapy, but the contribution of the shortening of the residence time of mefloquine to treatment outcome is an issue for further investigation. In this small study, however, no difference in mefloquine concentration profiles/kinetics between the patients with a sensitive response and those with a treatment failure was found.

The pharmacokinetics of dihydroartemisinin as an active metabolite of artemether or artesunate or an oral formulated drug have been defined in a few studies, with different routes of drug administration, disease stages and methods of drug analysis (Teja-isavadharm *et al,* 1996; Bernakis *et al*, 1997; Karbwang *et al,* 1997, 1998a,b; Na-Bangchang *et al*, 1998). This study was the first study that specifically addressed the pharmacokinetics of oral dihydroartemisinin when used in combination with mefloquine in patients with malaria. The high specificity and sensitivity of the HPLC-EC method used allowed the accurate characterization of dihydroartemisinin kinetics. Oral dihydroartemisinin is well absorbed in acute malaria. The high oral clearance value exceeding hepatic blood flow may imply that the drug is either incompletely absorbed from the gastro-intestinal tract (solubility/

Table 4
Pharmacokinetics of mefloquine in patients with uncomplicated falciparum malaria and healthy subjects*; data are presented as mean [95% CI] or median (95% CI) values.

Pharmacokinetic parameters	Healthy*	Regimen-I	Regimen-II	Regimen-III	Regimen-IV
C_{max} (ng/ml)**	1202 [1097-1305]	1728 [1492-1963]	1970 [1675-2264]	3249 [3075-3422]	2809 [2413-3205]
C_{max} (ng/ml per mg dose)	1.6 [1.47-1.74][a]	2.3 [1.98-2.61]	2.63 [2.23-3.01]	2.60 [2.46-2.74]	2.25 [1.93-2.56]
t_{max} (h)	4 (3.4-4.6)[b]	12 (10.6-16.6)	12 (11.7-19.7)	16 (12-16)[c]	48 (48-48)[d]
AUC (ng.h/ml)**	445 [378-513]	518 [421-614]	547 [462-632]	894 [800-988]	820 [704-935]
AUC (ng.h/ml per mg dose)	0.59 [0.5-0.68]	0.69 [0.56-0.82]	0.73 [0.62-0.84]	0.72 [0.64-0.79]	0.66 [0.56-0.75]
$AUC_{0\text{-}day1}$ (ng.h/ml per mg dose)	0.035 [0.023-0.04][e]	0.039 [0.031-0.045]	0.040 [0.039-0.052]	0.044 [0.041-0.049][f]	0.038 [0.026-0.029]
$AUC_{0\text{-}day7}$ (ng.h/ml per mg dose)	0.167 (0.141-0.213)[g]	0.275 (0.232-0.315)	0.278 (0.259-0.344)	0.246 (0.229-0.278)	0.246 (0.207-0.281)
$t_{1/2,z}$ (h)	16.4 [12.9-19.9][h]	10.9 [9.8-11.9]	10.4 [8.6-12.3]	11.9 [10.9-12.9]	11.5 [10.4-12.6]
MRT (h)	21.9 [17.9-26.0][i]	15.7 [14.2-17.2]	14.7 [12.4-17.1]	15.8 [14.2-17.4]	15.5 [14.0-17.0]
V_z/F (l/kg)	15.39 (13.82-19.7)[j]	9.0 (8.24-9.90)	8.7 (7.1-11.96)	10.52 (9.63-12.51)	10.04 (9.78-11.41)
CL/F (ml/min/kg)	0.57 (0.40-0.65)	0.39 (0.34-0.47)	0.41 (0.36-0.62)	0.49 (0.37-0.59)	0.49 (0.42-0.59)

*Na-Bangchang *et al,* 1999a
**Statistical test was not performed
[a] = significantly different from patients with regimen-I (p=0.00063, 95% CI -1.1, -0.29) , regimen-II (p=0.000004, 95% CI -1.43,-0.61), regimen-III (p=0.000006, 95% CI -1.4, -0.59), and regimen-IV (p=0.0014, 95% CI -1.05, -0.23);
[b] = significantly different from patients with regimen-I (p=0.00015, 95% CI -14, -6) , regimen-II (p=0.00016 95%CI -8,-6), regimen-III (p=0.000186, 95% CI -12,-8, and regimen-IV (p=0.00016, 95% CI -44, -44);
[c] = significantly different from patients with regimen-II (p=0.023, 95% CI -6, 0);
[d] = significantly different from patients with regimen-I (p=0.006, 95% CI -38, -30) , regimen-II (p=0.0025, 95%CI -38, -36), and regimen-III (p=0.00016, 95% CI -36, -32);
[e] = significantly different from patients with regimen-II (p=0.038, 95% CI 0, -0.019) , regimen-III (p=0.021, 95% CI -0.003, -0.016), and regimen-IV (p=0.028, 95% CI 0.001, 0.014;
[f] = significantly different from patients with regimen-I (p=0.049, 95% CI 0, 0.18) , regimen-II (p=0.0019, 95% CI 0.013, 0.021), and regimen-IV (p=0.00021, 95% CI 0.013, 0.02);
[g] = significantly different from patients with regimen-I (p=0.0041, 95% CI -0.139, -0.07) , regimen-II (p=0.00088, 95% CI -0.153, -0.0177), regimen-III (p=0.012, 95%CI 0.0108, 0.051, and regimen-IV (p=0.0015, 95%CI 0.0117, 0.043);
[h] = significantly different from patients with regimen-I (p=0.00033, 95% CI 2.66, 8.36) , regimen-II (p=0.000066, 95% CI 3.07, 8.78), regimen-III (p=0.0015, 95%CI 1.6, 7.31), and regimen-IV (p=0.00065, 95% CI 2.0, 7.7);
[i] = significantly different from patients with regimen-I (p=0.00084, 95% CI 2.71, 9.7) , regimen-II (p=0.000079, 95% CI 3.66, 10.64), regimen-III (p=0.00047, 95% CI 2.64, 9.63, and regimen-IV (p=0.00029, 95% CI 2.92, 9.91);
[j] = significantly different from patients with regimen-I (p=0.00016, 95% CI 4.8, 8.64) , regimen-II (p=0.0012, 95%CI 4.3, 8.96), regimen-III (p=0.00088, 95% CI 2.44, 6.39), and regimen-IV (p=0.0126, 95% CI 1.47, 5.85);
[k] = significantly different from patients with regimen-I (p=0.002, 95% CI -3.66, -0.96), and regimen-II (p=0.023, 95% CI -4.01, -0.57);
[l] = significantly different from patients with regimen-I (p=0.0015, 95% CI -5.28, -1.08) , and regimen-II (p=0.013, 95% CI -5.41, -0.94)

dissolution problems), and/or the drug undergoes an extensive first-pass metabolism.

Uncomplicated falciparum malaria also influenced the kinetics of oral dihydroartemisinin but probably by different underlying mechanism(s) from that seen with mefloquine. The influence of malaria on the overall kinetics of dihydroartemisinin could be attributable to the improvement of oral bioavailability of dihydroartemisinin, and/or impairment of hepatic clearance, and/or contraction of the apparent volume of distribution of the drug. Contraction of

the apparent volume of distribution of dihydroartemisinin could be a consequence of increased binding of the drug to plasma protein(s) or substantial accumulation of the drug in parasitized erythrocytes (Gu *et al,* 1984), although the latter seems more likely as the degree of binding of dihydroartemisinin to plasma protein is not extensive (40-50%) (Luo and Shen, 1987). Little is known about the ultimate fate of dihydroartemisinin in the human body. The *in vitro* studies using rat isolated perfused liver (IPRL) and microsomes have identified the glucuronide conjugate as a sole and principal metablite of dihydroartemisinin (Maggs *et al,* 1997; Batty *et al,* 1998). The extent of the hepatic extraction of oral dihydroartemisinin is unknown. If the extent of hepatic extraction of the drug is not extensive (low to intermediate), then the simultaneous prolongation of the elimination half-life and mean residence time observed would be associated with the reduced metabolic clearance of the drug during acute malaria. In support of our finding in humans, a recent animal study showed a significant reduction (by 20-30%) in the intrinsic metabolic clearance, together with biliary excretion of dihydroartemisinin-glucuronide conjugate (by 40-50%) in the *P. berghei* rodent malaria model (Batty *et al,* 1998). The alterations of the pharmacokinetics of oral dihydroartemisinin in malaria appear different from those observed with oral artmether and artesunate, of which impairment of oral absorption/bioabailability, and/or enhancement of their clearance are major kinetic changes (Karbwang *et al,* 1998a,b; Na-Bangchang *et al,* 1998). The differential influence of acute malaria infection on the kinetics of artemisinin derivatives is unexpected.

With respect to the clinical use of the combination dihydroartemisinin/ mefloquine, the potential for metabolic interactions between these two drugs is of concern and should be taken into account in the evaluation of its clinical efficacy. There is evidence of the ability of artemisinin to alter hepatic drug metabolism *in vivo* (Svensson *et al,* 1998). It is not known to what extent a similar capacity for induction also applies to other derivatives. Nonetheless, it was shown in our study that the pharmacokinetics of both dihydroartemisinin and mefloquine, when given concurrently on the same day or 24 hours apart, were similar (regimens-I and II). This probably excludes the possibility of pharmacokinetic interactions between these two drugs in patients with malaria. Delayed and marked inter-individual variability in the absorption kinetics in patients may have masked the effect of dihydroartemisinin in accelerating the oral absorption of mefloquine when given concurrently in healthy subjects (Karbwang *et al,* 1991). With other artemisinin derivatives (artesunate and artemether), their administration 6-24 hours after mefloquine resulted in significantly lower mefloquine concentrations in patients with malaria (Karbwang *et al,* 1994; Na-Bangchang *et al,* 1995a).

In general, the pharmacokinetics of mefloquine administered in various combination regimens with dihydroartemisinin were comparable. The only differences observed were the delayed time to reach maximum concentration and the enhanced systemic availability during the first day of mefloquine dosing in regimens with the two-divided, high dose mefloquine regimens (regimens-III and IV). This might be explained by the influence of the dose regimen/ schedule (the time of administration of the split doses). Due to a relatively slow absorption, the time required to attain the maximum concentration (t_{max}) of mefloquine could be delayed when split doses are given, and in particular if the second dose of mefloquine was given after 24 hours (regimen IV). In previous studies, administering mefloquine as a single 1,250 mg or as two divided doses at 6 hour intervals resulted in similar whole blood mefloquine concentration-time profiles (Na-Bangchang *et al,* 1995a). In our large clinical trial, the clinical efficacy of both regimens was similar (regimen III *vs* regimen IV: 97% *vs* 96.3%). It was shown that when mefloquine was given concurrently with dihydroartemisinin on the first day (regimens-I and IV), a high incidence of adverse effects, particularly nausea and vomiting, were recorded. Patients who vomited within the first hour of drug administration had markedly low concentrations of mefloquine in their blood. However, results from the large trial (Na-Bangchang *et*

al, 1999b) indicate that approaches in which mefloquine doses were added to a single dose of dihydroartemisinin (on the first or second day) may have no significant impact on induction of adverse effects, especially nausea and vomiting.

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