Clinical efficacy and pharmacokinetics of artemisinin monotherapy and in combination with mefloquine in patients with falciparum malaria

M. HASSAN ALIN^{1,2}, M. ASHTON¹, C. M. KIHAMIA³, G. J. B.MTEY⁴ & A. BJÖRKMAN^{2,5}

¹Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, Biomedical Centre, Uppsala University, Uppsala, Sweden, ²Division of Infectious Diseases, Huddinge University Hospital, Huddinge, Sweden,

³Department of Parasitology and Entomology, Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania, ⁴Kibaha Designated District Hospital, Kibaha Education Centre, Kibaha, Tanzania and ⁵Division of Infectious Diseases, Danderyd Hospital, Danderyd, Sweden

- 1 The aim of this study was to assess the pharmacokinetics, clinical efficacy and safety of artemisinin alone and in combination with mefloquine.
- 2 Thirty-eight adults with symptomatic *Plasmodium falciparum* malaria were randomly assigned to receive either artemisinin (500 mg single dose followed by another 500 mg on day 1 and then 250 mg twice daily for 4 days) or artemisinin (500 mg single dose followed by 750 mg on day 1 and then 250 mg three times daily for one more day) in co-administration with mefloquine (250 mg three times daily for the first day). All drug administration was by the oral route. Patients were hospitalized at the Kibaha Designated District Hospital, Kibaha, Tanzania, for 6 days and a follow up for 3 weeks was performed.
- 3 Treatment with the artemisinin/mefloquine combination resulted in a shorter parasite clearance time (PCT) of 24 (22, 27; 95% confidence interval) h *vs* 31 (27, 36) h and fever subsidence time (FST) of 14 (12, 16) h *vs* 20 (18, 23) h compared with artemisinin monotherapy. The 95% CI for the difference of the PCT and FST were 1.7, 12 and 3, 10, respectively. Parasites were detected in 7 out of 17 patients (41%) receiving artemisinin monotherapy at the 3rd and 4th week follow up visits. No parasites were detected after the combination therapy.
- 4 The maximum plasma concentrations ($C_{\max}$) were similar after artemisinin monotherapy (615.4 ± 387.0 ng ml⁻¹) and in combination with mefloquine (851.8 ± 523.6 ng ml⁻¹). Elimination half-lives ($t_{1/2}$) were also identical at 2.2 ± 0.6 h and 2.5 ± 0.7 h, respectively. However, the AUC values were higher ($P < 0.05$) after combination therapy (3252 ± 1873 ng ml⁻¹ h) than after monotherapy (2234 ± 1502 ng ml⁻¹ h). The oral clearance values were lower ($P < 0.05$) after combination therapy (195.4 ± 86.9 l h⁻¹) than after monotherapy (314.3 ± 189.4 l h⁻¹). PCT and FST normalized to initial parasitaemia correlated with AUC(0, t) ($r_s = 0.56$, $P = 0.02$, $r_s = 0.58$, $P = 0.01$, respectively) and with $C_{\max}$ ($r_s = 0.62$, $P = 0.01$, $r_s = 0.68$, $P = 0.005$, respectively) in the artemisinin monotherapy only.
- 5 One patient on the combination therapy developed a psychiatric condition and two patients on the monotherapy developed skin itch.

Keywords *Plasmodium falciparum* malaria artemisinin mefloquine efficacy pharmacokinetics

Correspondence: Dr M. Hassan Alin, Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, Box 580, Biomedical Centre, Uppsala University, 751 23 Uppsala, Sweden

Introduction

The endoperoxide sesquiterpene lactone, artemisinin and its derivatives have become increasingly important as antimalarial drugs with rapid action against multidrug resistant forms of *Plasmodium falciparum* malaria. Extensive clinical studies with artemisinin and its derivatives conducted mainly in Southeast Asia have shown a rapid effect but with a relative high recrudescence rate.

Combinations of artemisinin with mefloquine, primaquine or tetracycline have shown potentiation *in vitro* [1] and in rodent malaria [2]. In Burma, cerebral malaria patients were treated with either artemether intramuscularly (600 mg over 3 days) followed by oral mefloquine (1000 mg as a single dose), intravenous artesunate (300 mg over 3 days) followed by mefloquine (1000 mg) or quinine (1800 mg) in combination with tetracycline capsules (1000 mg for 7 days) [3]. The overall mortality rates were 14%, 8% and 34%, respectively. Recrudescence was only observed after the quinine/tetracycline. In Thai patients with uncomplicated *P. falciparum* randomized to artesunate (600 mg over 5 days), mefloquine (1250 mg in two divided doses) or artesunate followed by mefloquine, the cure rates for mefloquine and artesunate alone were respectively 81% and 88% while the combination yielded a radical treatment [4]. In three additional studies, artemisinin or artesunate combined with mefloquine produced clearance of parasites but with up to 15% recrudescence rate [5–7]. There are few data on the efficacy of artemisinin and its derivatives in Africa.

Pharmacokinetic data on artemisinin and its derivatives are scarce. Artemisinin (400 mg) given orally to healthy subjects was rapidly absorbed with mean absorption time of 0.78 h [8]. After a 500 mg oral dose to healthy subjects a mean maximum concentration (C_{max}) of 391–405 ng ml⁻¹ occurred at 1.5–1.8 h after drug intake [9, 10].

Pharmacokinetic information about artemisinin and its derivatives is limited and particularly so in patients. There is potential advantage of combining artemisinin with mefloquine in reducing the dose and treatment period of artemisinin and its derivatives which will improve compliance in clinical practice. However, information on pharmacokinetic interaction during combination therapy and its consequence for efficacy and safety has not been fully explored. This study was performed to assess pharmacokinetics of artemisinin monotherapy and in combination with mefloquine as well as to compare the clinical efficacy and safety of artemisinin monotherapy for 6 days with a short course of artemisinin administered simultaneously with mefloquine.

Methods

Ethical approval

The clinical trial was approved by the Research and Publication Committee of the Muhimbili University

College of Health Sciences, Dar es Salaam, Tanzania. Research clearance was obtained from the Tanzanian Commission for Science and Technology. The study was also approved by the Swedish Medical Products Agency and the ethics committee of the medical faculty, Uppsala University, Uppsala, Sweden. Informed consent was obtained from all patients prior to participation in the study.

Patients

The trial was designed as an open randomized comparative study of artemisinin capsules alone or in combination with mefloquine. The study was conducted at the Kibaha Designated District Hospital, 40 km northwest of Dar es Salaam, Tanzania, between May and July 1994. The area is holoendemic for malaria with high transmission from March to June and from October to December. Patients between 15 to 45 years of age with ≥ 1000 parasites per μ l capillary blood, verified as *Plasmodium falciparum* by light microscopy, and body temperature above 37.5°C were included in the trial. Severe or cerebral malaria, recent treatment with antimalarial drugs and pregnancy were exclusion criteria. All patients were hospitalized for 6 days and were then seen once weekly for an additional 3 weeks.

Drug regimens

Eighteen patients received artemisinin alone [treatment A] while 20 patients received artemisinin in combination with mefloquine [treatment A+M]. Treatment A started with a single dose of 500 mg followed by another 500 mg on day 1 and then 250 mg twice daily for 4 days (total dose=3000 mg). Treatment A+M was 500 mg single dose artemisinin followed by 750 mg on day 1 and then 250 mg every 8 h for one more day (total dose=2000 mg). Mefloquine (250 mg) was co-administered with artemisinin every 8 h during the first day (total dose=750 mg). Drug administration was by oral route. Artemisinin capsules were obtained from the Institute of Materia Medica, Department of Production, Hanoi, Vietnam. Mefloquine, Lariam® (Roche, Switzerland) was received from the University Hospital, Uppsala, Sweden.

Clinical and parasitological assessment

Thick smears were prepared from capillary blood prior to treatment then every 3 h for the first 33 h and thereafter twice daily for 6 days. After hospital discharge of patients, thick smears were taken during the follow up visits on Days 14, 21 and 28. The smears were stained with Giemsa and parasitaemia determined by light microscopy against 200 white blood cells (WBC). Parasite density expressed as the number of parasites per microliter blood was obtained by multiplying with the mean WBC count of the individual patients determined on Days 1, 6 and 14. Thick films were

considered negative for parasites after reading three consecutive slides. The microscopist was blinded to treatment arms. Sublingual temperature was monitored in conjunction with sampling for the determination of parasitaemia.

Full haematology, serum biochemistry and urine were examined before drug intake and on Days 6 and 14. Haematological and urine investigations were conducted at Kibaha Hospital. Serum biochemistry tests were performed at Muhimbili Medical Centre, Dar es Salaam. Blood pressure and pulse rate were recorded prior to drug administration, twice daily during the admission period and once during each follow up visit. Clinical signs and symptoms were recorded before drug intake, once per day during the hospitalisation period and again during the follow up visits. Patients were interviewed about adverse events once daily during the hospitalisation and at the time of follow up visits. The interviews were open at first followed by specific questions according to a check list.

Pharmacokinetics

Blood (7 ml) was drawn into a heparinized Vacutainer from a forearm vein at the following times: -0.5, 0.5, 1, 1.5, 2, 2.5, 3.5, 4.5, 6 and 8 h. The blood samples were placed at 4°C for 15 min and then centrifuged at 3000 rev min⁻¹ for 10 min at ambient temperature. The plasma samples were immediately stored at -40°C at the Kibaha Designated District Hospital. The samples were transported to Uppsala in dry ice and stored at -80°C until analysis within 5 months. Artemisinin plasma concentrations were measured using h.p.l.c. method with post-column derivatisation and u.v. detection [11]. Standard curves were linear in the range of 10–2000 ng ml⁻¹, which encompassed the observed plasma concentrations. Six quality control samples, two each at 51, 245 and 1059 ng ml⁻¹ were analysed with each run. The absolute recovery from spiked plasma samples was more than 95% in the concentration range of 10–500 ng ml⁻¹. The limit of quantitation was 10 ng ml⁻¹ (CV = 12%). The intra-assay variability was 13% at 10 ng ml⁻¹ (n = 9) and 4% at 500 ng ml⁻¹ (n = 9). The inter-assay variability ranged from 9.6% (51 ng ml⁻¹) to 8.5% (1059 ng ml⁻¹). Quality control samples were 103, 106 and 102% of the theoretical (51, 245 and 1059 ng ml⁻¹).

Data analysis

Parasite clearance time (PCT) was taken as the time from initiation of therapy to the first negative blood smear which remained negative for three consecutive slides. PC₅₀ was defined as the time for 50% reduction of initial parasite density and was determined by linear interpolation from plots of parasitaemia *vs* time. Fever subsidence time (FST) was the time required for the body temperature to fall below 37.5°C and remain so for three consecutive readings.

Pharmacokinetic parameters were calculated by using

non-compartmental methods. The terminal elimination rate constant (λ_z) was estimated by log-linear regression of the terminal 3, 4 or 5 plasma concentration-time points. The elimination half-life ($t_{1/2}$) was calculated from log-linear regression of at least three last plasma concentration-time data. The areas under the curve from zero time to the last observed time (AUC 0, 8 h) was calculated by linear trapezoidal rule for ascending data points and by log trapezoidal rule for descending data points. AUC, the AUC extrapolated from the last data point to infinity was estimated by dividing the regressed concentration at the last time point by the estimated elimination rate constant. Oral clearance was obtained by Dose/AUC (=CL/F). The steady state volume of distribution uncorrected for the extent of bioavailability (V_{ss}/F) was calculated as Dose · AUMC/AUC². The time at which maximum concentrations occurred (t_{max}) and the maximum concentrations (C_{max}) were obtained directly from the plasma concentration-time data.

Statistical evaluation

Pharmacokinetic parameters between the two treatment arms were compared by Mann-Whitney U test. Initial parasite density, demographic data, PCT, PC₅₀ and FST were compared between the two treatment arms by 95% confidence intervals for the difference of the means. Haematology and blood biochemistry variables were compared by one-way ANOVA. Correlations were sought between initial parasitaemia to PCT, PC₅₀, and FST; PCT to FST and initial temperature to FST by linear regression. PCT, and FST normalized to initial parasitaemia were correlated with the AUCs or C_{max} values by Spearman's test. The level of significance was set to ≤0.05 in all statistical analysis.

Results

Baseline characteristics (weight, age and initial parasite density) were similar for the two treatments (Table 1).

Artemisinin alone and in combination with mefloquine had a rapid clinical effect (Table 1, Figures 1a and 1b). On average, parasite clearance time occurred 7 (95% CI difference 1.7, 12) h earlier after combination treatment compared with after single artemisinin treatment. Similarly, fever subsidence times occurred on average 6 (95% CI difference 3, 10) h earlier after treatment A + M compared with after treatment A. The PC₅₀ values were similar in both treatment arms. Significant positive correlations were observed between PCT and FST ($r^2 = 0.78$, $P < 0.001$, for treatment A and $r^2 = 0.31$, $P = 0.01$ for treatment A + M). Initial parasite density correlated with PC₅₀ ($r^2 = 0.34$, $P < 0.001$) but not with PCT or FST whether all the data were pooled or each group tested separately.

For treatment A and treatment A + M, respectively, 17/18 (94%) and 17/20 (85%) completed the follow up visits (Table 1). Seven out of the 17 patients who

Table 1 Mean (95% confidence interval) demographics and clinical efficacy for patients receiving artemisinin monotherapy (treatment A) or in combination with mefloquine (treatment A + M). 95% confidence interval for the mean differences of the demographic data and therapeutic parameters are given

	Treatment A	Treatment A + M	Difference
Patients (males/females)	18 (13 M/5 F)	20 (15 M/5 F)	
Age (years)	21.8 (18.7, 24.8)	21.4 (18.9, 23.7)	0.4 (−3.5, 4.4)
Weight (kg)	53.8 (50.4, 57)	55 (51.6, 58.3)	−1.2 (−6, 3.5)
Initial parasitaemia/µl blood	9803 ^a (7673, 18480)	10589 ^a (9201, 18569)	−806 (−7161, 5550)
PCT (h)	31.2 (26.8, 35.5)	24.3 (21.9, 26.7)	6.9 (1.7, 12)
PC ₅₀ (h)	10.8 (8.8, 12.7)	9.4 (7.8, 11)	1.4 (−1.2, 4)
FST (h)	20.2 (17.5, 22.9)	13.8 (11.9, 15.7)	6.4 (3, 10)
Recrudescence/reinfection	7 (41%)	0	
Completed the follow ups	17/18 (94%)	17/20 (85%)	

^aGeometric mean.

Figure 1a) Individual parasites per µl capillary blood *vs* time in 18 symptomatic falciparum malaria patients following oral artemisinin administration alone for 6 days and b) Individual parasites per µl capillary blood *vs* time in 20 symptomatic falciparum malaria patients following oral artemisinin administration for 2.5 days in combination with mefloquine on Day 1.

completed all follow up visits (41%) of treatment A got reinfection/recrudescence. In three patients, parasites were detected at the 21-day follow up visit and in four patients at the 28-day visit. These patients were treated with three tablets of Fansidar® (Roche). Artemisinin concentration-time profiles are shown in Figure 2. The calculated pharmacokinetic parameters are presented in Table 2. In three patients from each treatment arm there were not enough data to fully

Figure 2 Artemisinin plasma concentrations *vs* time (mean $\pm$ s.e. mean after 500 mg oral dose of artemisinin alone (○, *n* = 18) or in combination with mefloquine (△, *n* = 20) in adult patients with symptomatic falciparum malaria.

describe the elimination phase of the plasma concentration-time profile. For these six patients AUC(0, 8 h), *C*_{max} and *t*_{max} were computed. The *C*_{max}, *t*_{max} and *t*_{1/2} values of artemisinin were similar in both treatments. The AUC values after treatment A were lower and the *CL*_{oral} and the *V*_{ss}/*F* values higher compared to treatment A + M (Table 2). Pharmacokinetic parameters of artemisinin were found to be similar in male and female patients (Student's *t*-test) although only 10 females were included in the study. In the monotherapy group, PCT and FST normalized to initial parasitaemias correlated with AUC(0, 8 h) values (*r*_s = 0.56, *P* = 0.02 and *r*_s = 0.58, *P* = 0.01, respectively) and with *C*_{max} values (*r*_s = 0.62, *P* = 0.01 and *r*_s = 0.68, *P* = 0.005, respectively). The PCT and FST normalized to initial parasitaemias did not correlate with any artemisinin kinetic parameter in the combination therapy group. Non-normalized effect variables (PCT, PC₅₀ and FST) did not correlate with any kinetic parameter. Erythrocyte count and haemoglobin concentration were below the normal laboratory ranges in 10 and 9 patients respectively prior to drug administration in

Table 2 Mean $\pm$ s.d. (range) artemisinin pharmacokinetic parameters after the first 500 mg oral administration to falciparum malaria patients who received artemisinin alone (treatment A) or in combination with mefloquine (treatment A + M)

	treatment A (n = 18)	treatment A + M (n = 20)	P value
AUC(0, 8 h) (ng ml ⁻¹ h)	2014 $\pm$ 1359 (505.7–5502)	2786 $\pm$ 1608 (1096–6894)	<0.05
AUC (ng ml ⁻¹ h)	2234 $\pm$ 1502 ^a (675.9–6412)	3252 $\pm$ 1873 ^b (1337–7409)	<0.05
<i>t</i> _{1/2} (h)	2.2 $\pm$ 0.6 ^a (1.1–3.4)	2.5 $\pm$ 0.7 ^b (1.5–4.0)	NS
CL/F (1 h ⁻¹)	314.3 $\pm$ 189.4 ^a (78.00–740.0)	195.4 $\pm$ 86.91 ^b (67.00–374.0)	<0.05
<i>V</i> _{ss} /F (1)	1578 $\pm$ 986.7 ^a (403.0–3822)	976.8 $\pm$ 464.9 ^b (292.0–2095)	<0.05
<i>C</i> _{max} (ng ml ⁻¹)	587.4 $\pm$ 385 (99.4–1602)	818.3 $\pm$ 493.1 (262.1–2073)	NS
<i>t</i> _{max} (h)	2.5 (1.0–4.5)	2.0 (1.0–6.0)	NS

NS = not significant. ^an = 15. ^bn = 17.

both treatment arms. Serum transaminases and total bilirubin levels were above the normal laboratory ranges in 5 and 12 patients respectively before drug intake in both treatment arms. All variables normalized on Day 6 and were consistent on Day 14. Blood pressure (systolic and diastolic) and pulse rate monitored before drug administration, daily during the hospitalisation and once at each follow up visit were consistently normal. One female patient in the combination therapy developed a psychiatric condition, including restlessness, dysphoria, lack of concentration and insomnia. The symptoms developed 1 week after initiation of treatment and resolved spontaneously 2 weeks later. Two male patients treated with artemisinin alone developed skin itch on the upper and lower extremities which disappeared 1 day before discharge. No treatment was required.

Discussion

Artemisinin and its derivatives have shown a rapid effect against *Plasmodium falciparum* malaria but are associated with high frequency of recrudescence. Artemisinin regimens of more than 5 days duration have a lower incidence of recrudescence rate [12] though with a reduced compliance to be expected in clinical practice. We have compared the clinical efficacy of oral artemisinin alone treatment for 6 days with a short course of artemisinin in combination with mefloquine as well as artemisinin pharmacokinetics.

All patients in both treatment arms cleared parasitaemia and were free from symptoms almost within 1 day. The co-administration of artemisinin and mefloquine

resulted in shorter parasite clearance and fever subsidence times than artemisinin alone possibly reflecting a potentiative antimalarial activity which has earlier been observed *in vitro* and in rodent models [1, 2]. Potentiation has not been reported in earlier clinical studies [3–5] and may be due to non-concurrent dosing unlike in the present study where mefloquine and artemisinin were initiated simultaneously. In addition, in our study, effect variables were monitored more frequently than in the previous studies cited. The artemisinin dose in the combination treatment arm on day 1 was slightly higher than the monotherapy treatment arm. Since previous studies have shown that the rapid effect of artemisinin compounds is not related to the strength of initial doses [13–16] we consider it unlikely that the shorter parasite clearance time in the combination therapy is due to the difference in dose.

In one *in vitro* study performed in the same study locality as this study, the EC₅₀ values of mefloquine were higher than artemisinin and chloroquine [17]. The median EC₅₀ values increased in the order: artemisinin < chloroquine < mefloquine.

The combination therapy in the present study resulted in a radical cure while the monotherapy regimen was associated with 41% reinfection/ recrudescence rate. Parasites were detected in these patients at the third or fourth week follow up visits. Artemisinin pharmacokinetic parameters in these patients did not differ from the rest of patients. Since this study was conducted in a holoendemic area for malaria it was not possible to distinguish recrudescence from reinfection. It is likely that at least some of these parasitaemias may have been due to reinfection as they were detected as late as the third and fourth week follow up visits.

Pharmacokinetic parameters determined in this study were similar to those previously reported in healthy subjects [8–10]. This suggests that uncomplicated *Plasmodium falciparum* malaria does not influence the kinetics of artemisinin after a single dose administration. Artemisinin pharmacokinetic parameters, however, displayed marked interindividual variability in contrast to one previous study in a limited number of healthy subjects [9]. Such interindividual variability in our study might be due to the malaria infection. The absolute bioavailability (*F*) for artemisinin is unknown. *In vitro* experiments with rat liver tissues have demonstrated that artemisinin was rapidly metabolized by the liver [18]. The CL_{oral} values found in the present study are high suggesting a low bioavailability after oral administration. In our study, the elimination half-life based on plasma concentrations in patients was short. From a pharmacokinetic point of view, it is justifiable to administer artemisinin at least twice daily.

The AUC values were higher and CL_{oral} and *V*_{ss}/*F* values lower in the combination therapy than the monotherapy. The change in AUC, CL_{oral} and *V*_{ss}/*F* suggests an increased bioavailability of artemisinin may be due to a metabolic interaction with mefloquine in the gut or in the liver. It has been shown earlier that artemether or artesunate did not significantly inhibit carboxymefloquine formation from mefloquine in human liver microsomes [19]. However, mefloquine

blood concentrations were lower when mefloquine was co-administered with artesunate which was interpreted as a protein binding interaction [20].

Erythrocyte count and haemoglobin concentration before drug intake were below the normal laboratory range in some patients. Serum transaminases and total bilirubin levels were above the normal laboratory range in some patients prior to drug administration. This change of blood variables might have been due to the malaria infection. All variables normalized on Day 6. Blood pressure (systolic and diastolic) and pulse rates were normal in both treatment arms. One female patient receiving the combination therapy developed a psychiatric condition. Symptoms developed during the hospitalisation period and resolved spontaneously after 2 weeks. Psychiatric disorders have been associated with mefloquine intake [21]. In addition, skin itch developed on the extremities in two male patients treated with artemisinin alone during the hospitalisation period but did not require specific treatment. Itching related to artesunate has been reported earlier [4].

In conclusion, artemisinin AUC was higher and oral clearance lower in the combination therapy than the monotherapy, suggesting a drug interaction. The combination therapy resulted in a more rapid clearance of parasitaemia than artemisinin alone and produced a radical cure. This combination therapy reduces the dose and duration of artemisinin treatment and will thus improve compliance. However, the relative high cost of mefloquine and its association with psychiatric disorders even at reduced doses may limit the acceptability of this combination for the treatment of malaria.

We wish to thank Lars H. Norvik, the Swedish International Development Agency (SIDA), Dar es Salaam, Tanzania for administrative facilities. We are grateful to Nurse Jeannette Daal for technical assistance. The authors are indebted to the staff of the Kibaha Designated District Hospital for their skillful assistance. The study received financial support from the Swedish Agency for Research Co-operation with Developing Countries (SAREC).

References

- Chawira AN, Warhurst DC. The effect of artemisinin combined with standard antimalarials against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* in vitro. *J Trop Med Hyg* 1987; **90**: 1–8.
- Chawira AN, Warhurst DC, Robinson BL, Peters W. The effect of combinations of qinghaosu (artemisinin) with standard antimalarial drugs in the suppressive treatment of malaria in mice. *Trans R Soc Trop Med Hyg* 1987; **81**: 554–558.
- Win K, Than M, Thwe Y. Comparison of combinations of parenteral artemisinin derivatives plus oral mefloquine with intravenous quinine plus tetracycline for treating cerebral malaria. *Bull WHO* 1992; **70**: 777–782.
- Looareesuwan S, Viravan C, Vanijanonta S, et al. Randomized trial of artesunate and mefloquine alone and in sequence for acute uncomplicated falciparum malaria. *Lancet* 1992; **339**: 821–824.
- Looareesuwan S, Kyle DE, Viravan C, et al. Treatment of patients with recrudescence falciparum malaria with a sequential combination of artesunate and mefloquine. *Am J Trop Med Hyg* 1992; **47**: 794–799.
- Looareesuwan S, Viravan C, Vanijanonta S, et al. Treatment of acute uncomplicated falciparum malaria with a short course of artesunate followed by mefloquine. *Southeast Asian J Trop Med Public Health* 1993; **24**: 230–234.
- Hien TT, Arnold K, Hung NT, et al. Single dose artemisinin-mefloquine treatment for acute uncomplicated. *Trans R Soc Trop Med Hyg* 1994; **88**: 688–691.
- Titulaer H A C, Zuidema J, Kager P A, et al. The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers. *J Pharm Pharmacol* 1990; **42**: 810–813.
- Duc DD, De Vries PJ, Khanh NX, et al. The pharmacokinetics of a single dose of artemisinin in healthy Vietnamese subjects. *Am J Trop Med Hyg* 1994; **5**: 785–790.
- Loutan L, Paris M, Plessas C, et al. Pharmacokinetics of artemisinin after oral administration in healthy volunteers. *Am J Trop Med Hyg* 1993; **49** abstract 281.
- Edlund P O, Westerlund D, Carlqvist J, et al. Determination of artesunate and dihydroartemisinin in plasma by liquid chromatography with post-column derivatization and UV-detection. *Acta Pharm Suec* 1984; **21**: 223–234.
- Li GQ, Guo XB, Fu LC, et al. Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. *Trans R Soc Trop Med Hyg* 1994; **88**: 5–6.
- Bunnag D, Viravan C, Looareesuwan S, et al. Clinical trial of artesunate and artemether on multidrug resistant falciparum malaria in Thailand. *Southeast Asian J Trop Med Public Health* 1991; **22**: 380–385.
- Bunnag D, Viravan C, Looareesuwan S, et al. Double blind randomized clinical trial of oral artesunate once or twice daily dose in falciparum malaria. *Southeast Asian J Trop Med Public Health* 1991; **22**: 539–543.
- Bunnag D, Karbwang J, Harinasuta T. Artemether in the treatment of multiple drug resistant falciparum malaria. *Southeast Asian J Trop Med Public Health* 1992; **23**: 762–767.
- Sy ND, Hoan DB, Dung NP, et al. Treatment of malaria in Vietnam with oral artemisinin. *Am J Trop Med Hyg* 1993; **48**: 398–402.
- Hassan Alin M, Kihamia C M, Björkman A, et al. Efficacy after oral and intravenous artesunate in male Tanzanian adults with *Plasmodium falciparum* malaria and in vitro susceptibility to artemisinin, chloroquine and mefloquine. *Am J Trop Med Hyg* 1995; **53**: 639–645.
- Niu Xinyi, Ho Liyi, Ren Zhihong, Song Zhenyu. Metabolic fate of Qinghaosu in rats; a new TLC densitometric method for its determination in biological material. *Eur J Drug Metab Pharmacokinet* 1985; **10**: 55–59.
- Na Bangchang K, Karbwang J, Back DJ. Mefloquine metabolism by human liver microsomes, effect of other antimalarial drugs. *Biochem Pharmacol* 1992; **43**: 1957–1961.
- Karbwang J, Na Bangchang K, Thanavibul A, et al. Pharmacokinetics of mefloquine when given alone and in combination with artesunate. *Bull WHO* 1994; **72**: 226–229.
- Patchen JH, Campbell CC, Williams SB. Neurological reactions after a therapeutic dose of mefloquine. *N Engl J Med* 1989; **325**: 1415–1416.

(Received 6 July 1995,
accepted 24 January 1996)