

# Falciparum malaria in eastern Thailand: a randomized trial of the efficacy of a single dose of mefloquine

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Reported are the results of a randomized trial of a single dose of mefloquine (15 mg/kg or 25 mg/kg body weight) for the treatment of uncomplicated multidrug-resistant falciparum malaria. Of the 110 adult patients enrolled in the study 57 were randomly assigned to the 15 mg/kg group and 53 to the 25 mg/kg group. The baseline characteristics of the patients did not differ significantly in the two groups, except that those in the 15 mg/kg group had lower haemoglobin levels. Adverse effects following treatment were commoner in the 25 mg/kg group, but not significantly so.

Seven patients (6%) did not complete the 42-day follow-up. The parasitological failure rates in the 15 and 25 mg/kg groups were, respectively, 50% (28/56) and 43% (23/53) on day 28, and 62% (33/53) and 56% (28/50) on day 42. Treatment failures were not correlated with the serum mefloquine concentrations on day 2, and 13 out of 19 patients with serum mefloquine concentrations >2000 µg/l on day 2 showed an R response during the follow-up. The mean ratio between the concentrations of the (SR)-(-) and (RS)-(+) enantiomers of mefloquine on day 2 was 3.37, indicating that there are differences in their pharmacokinetics.

Re-treatment of patients who showed an R response with seven days of quinine (30 mg.kg<sup>-1</sup>.day<sup>-1</sup>)+ tetracycline (25 mg.kg<sup>-1</sup>.day<sup>-1</sup>) was successful in 93% of the cases.

## Introduction

Mefloquine has been used since 1984 to treat uncomplicated falciparum malaria in Thailand. To delay the development of mefloquine resistance in *Plasmodium falciparum* strains, mefloquine was at first given in a triple combination with sulfadoxine and pyrimethamine. At a dose of 15 mg per kg body weight, mefloquine produced cure rates close to 100% in field trials (1-4). However, because of the low susceptibility of *P. falciparum* strains to sulfadoxine and pyrimethamine, treatment centres serving Khmer displaced persons along the eastern border of

Thailand changed in July 1990 from the triple combination to mefloquine alone. A year later, an *in vivo* test of mefloquine efficacy in a Khmer displaced persons camp reported a cure rate of only 41% (5). Since it had been reported in an area adjacent to the Cambodian border that patients who received 25 mg of mefloquine per kg body weight had a higher maximum plasma mefloquine concentration than patients treated with 15 mg/kg (6), we initiated a clinical trial to compare the *in vivo* outcomes of patients treated with a single dose of 15 mg/kg or 25 mg/kg of mefloquine.

## Patients and methods

### Study site

The study was carried out in Site 8, a Khmer displaced persons camp on the border between Thailand and Cambodia. The camp had a population of 45 000, 40% of whom were children under the age of 10 years; it was also used as a referral hospital for patients from Cambodia. Malaria is the leading cause of morbidity and mortality in the camp, according to data from the outpatient department and the hospital. The monthly malaria incidence typically varies between 50 and 2000 cases, depending on the season. Most cases are due to *P. falciparum* (90%), the

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remainder being *P. vivax* infections. Uncomplicated falciparum malaria cases are treated with 15 mg/kg body weight of mefloquine (Lariam, Roche) in a single dose. Severe cases are treated with intravenous quinine for up to 7 days, which is replaced by orally administered quinine and tetracycline when the patient is able to swallow tablets.

Mosquito collections made over the period 1983–85 show that areas close to the camp were heavily infested with both of the region's malaria vectors, *Anopheles dirus* and *A. minimus*.

### Study procedure

Between September and November 1991, patients diagnosed with uncomplicated falciparum malaria at the outpatient department were treated with the camp's usual regimen of a single dose of 15 mg/kg of mefloquine. The following were contraindications for mefloquine treatment: cerebral symptoms, haemoglobin level <7 g/dl, high parasitaemia, and pregnancy.

Immediately after this first dose, adults with no history of treatment with quinine or mefloquine during the previous month were recruited for the study if they were willing to be randomized to receive a further dose of 10 mg/kg of mefloquine and if they were willing to remain available for follow-up for 42 days. The informed consent to participate in the study was obtained from the patients through the help of a translator, and the dose of 10 mg/kg of mefloquine was given to patients chosen using a table of random numbers. The interval between the first and the second dose never exceeded 15 minutes. Routine clinical information was collected using a standard form while the patients remained under observation for one hour after treatment in order to record whether or not they vomited within this period.

All thick smears were subsequently reviewed by an expert microscopist from the Thai Malaria Division. The number of parasites was calculated against 100 leukocytes (assuming a leukocyte count of  $8.0 \times 10^9$  per litre). Patients were retained for the study if they had >1600 asexual forms of *P. falciparum* per  $\mu$ l of blood and no mixed *P. falciparum*/*P. vivax* infections.

Patients were asked to return to the outpatient department for a thick smear on days 2, 7, 14, 21, 28, 42 and if they experienced any fever or symptoms of malaria outside the regular days of follow-up.

Treatment responses were categorized as RI, RII, RIII, or sensitive, using a modification of the WHO classification scheme (7). (RI was assigned to all patients who had a negative slide on day 7 but whose slide became positive for asexual forms of *P. falciparum* between day 8 and day 42; RII, to patients whose parasitaemia on day 2 had decreased

by  $\geq 75\%$  of the level on day 0, and whose slides were still positive for asexual forms of *P. falciparum* on day 7; RIII, to patients whose parasitaemia on day 2 had decreased by <75% of that on day 0; and sensitive, to patients who remained free of parasitaemia after day 6. An "R" response is used later in this article to indicate either RI, RII, or RIII. All R responses were treated orally for 7 days with quinine (30 mg per kg per day) + tetracycline (25 mg per kg per day) in hospital. A final thick smear was taken 14 days after the first day of quinine treatment to confirm the cure.

After their further consent had been obtained, 29 patients were randomly selected and 5 ml of venous blood was collected from each of them on day 2 in order to determine their serum mefloquine concentration. Serum was separated from blood by centrifugation, kept on ice, and stored at <4 °C. The centrifugation was performed by hand and separation of serum may therefore have been incomplete for some blood samples. Mefloquine and its carboxylic metabolite in the serum sample were assayed using high-performance liquid chromatography (HPLC) (8) and the (*SR*)-(-) and (*RS*)-(+)-enantiomers of mefloquine by an enantioselective HPLC method using a chiral counter-ion (9). The lower limit of determination was 50  $\mu$ g/l for mefloquine and its carboxylic metabolite and 125  $\mu$ g/l for the individual enantiomers.

The study design was approved by the ethics committee of the United Nations Border Relief Operations (UNBRO).

**Statistical analysis.** Differences in proportions were analysed using  $\chi^2$  and Fisher's exact tests. Means were compared using Student's *t*-test.

## Results

### Patients' characteristics at baseline

A total of 110 patients were enrolled in the study from November to December 1991: 57 received 15 mg/kg body weight of mefloquine and 53 received 25 mg/kg. The patients' baseline characteristics are summarized in Table 1. There were no significant differences between the two groups, except for the mean haemoglobin level, which was lower for the 15 mg/kg group (9.4 g/dl versus 10.0 g/dl,  $P=0.03$ ).

### Adverse effects of the treatment

Of the 57 patients in the 15 mg/kg group, four (7%) vomited within one hour of receiving mefloquine, 11 (19%) reported diarrhoea in the first two days after treatment, and 40 (70%) complained of dizziness on day 2; among the 53 patients in the 25 mg/kg group, the corresponding numbers were 7 (13%), 14 (26%),

Table 1: Baseline characteristics of the 110 study patients

	Mefloquine dose (mg/kg body weight)	
	15	25
No. of patients	57	53
No. of males	49 (86) <sup>a</sup>	43 (81)
Mean age ± SD (years)	31.8 ± 11.0	34.7 ± 12.3
No. of reported mefloquine treatments in past year	0 1-2 ≥3	30 (53) 24 (45) 22 (42) 7 (13)
History of vomiting or diarrhoea prior to treatment	13 (23)	16 (30)
Mean haemoglobin level ± SD (g/dl)	9.4 ± 1.2	10.0 ± 1.5 <sup>b</sup>
Geometric mean parasitaemia (per µl)	25 600	34 900

<sup>a</sup> Figures in parentheses are percentages.

<sup>b</sup>  $P < 0.05$ , Student's *t*-test.

and 39 (74%). None of these differences was statistically significant. Patients who complained of digestive disorders at the initial examination were more likely to report a history of diarrhoea during the first two days after treatment ( $P = 0.02$ ).

Two patients in the 25 mg/kg group who vomited within one hour of receiving their dose were re-treated because it appeared that the drug had been regurgitated in the vomitus.

### Mefloquine failure

Of the 110 patients, seven (6%) did not complete the follow-up: four in the 15 mg/kg group (one lost to follow-up on day 15 and three on day 29); and three in the 25 mg/kg group (all lost to follow-up on day 29).

The parasitological failure rates on day 28 and day 42 for the two treatment groups are shown in Table 2. The proportion of RIII patients was lower in the 25 mg/kg group, but there was no overall impact of dose on the treatment failure rates.

A total of 50 of the 61 patients with an R response agreed to be treated orally for 7 days in hospital with quinine (30 mg.kg<sup>-1</sup>.day<sup>-1</sup>) + tetracycline (25 mg.kg<sup>-1</sup>.day<sup>-1</sup>). Of these patients, 45 returned for a control thick smear 14 days after the initiation of the quinine-tetracycline treatment: 42 (93%) patients had a negative smear; one had a positive smear on day 14, but a negative smear on day 16 and was then not re-treated; and the other two patients who were positive on day 14 were re-treated with quinine-tetracycline, an initial course of quinine being given intravenously.

### Serum mefloquine concentrations

A total of 29 patients, (16 in the 15 mg/kg group and 13 in the 25 mg/kg group) agreed to have samples of blood drawn on day 2 for measurement of the serum mefloquine concentration. The mean serum mefloquine concentration was almost identical in the two groups (2165 µg/l in the 15 mg/kg group versus 2284 µg/l in the 25 mg/kg group;  $P = 0.69$ ). The standard deviation (SD) of the serum mefloquine concentration was considerably greater for the 25 mg/kg group (995 µg/l versus 602 µg/l). The mean ± SD serum concentration on day 2 for the mefloquine enantiomers was 1689 ± 578 µg/l for (SR)-(-) and 535 ± 220 µg/l for (RS)-(+). The serum concentrations of the (SR)-(-) and (RS)-(+) enantiomers were strongly correlated with the total serum mefloquine concentrations ( $r = 0.99$  and  $0.92$  resp.). The mean ratio of the concentrations of the (SR)-(-) and (RS)-(+) enantiomers on day 2 was 3.37.

Patients who vomited within an hour of treatment had significantly lower serum mefloquine concentrations on day 2 than those who did not (mean: 1289 µg/l versus 2300 µg/l;  $P = 0.03$ ). Serum mefloquine levels were the same for the seven patients who had a history of diarrhoea during the first two days after treatment as those who did not (mean: 2073 µg/l versus 2264 µg/l;  $P = 0.58$ ). Patients who complained of dizziness on day 2 had a higher serum mefloquine concentration than those who did not (mean: 2394 µg/l versus 1371 µg/l;  $P = 0.006$ ).

There was no correlation between the *in vivo* outcome and the serum mefloquine concentration on day 2: patients who showed an R response had a mean serum concentration of 2344 µg/l compared with 2012 µg/l for patients who were successfully treated ( $P = 0.33$ ). It should be noted that 13 of the 19 patients whose serum mefloquine concentration was >2000 µg/l on day 2 exhibited an R response. Patients with RII and RIII responses had lower

Table 2: Mefloquine response rate in the two treatment groups

	Day of follow-up:			
	28		42	
	Mefloquine dose (mg/kg body weight)		Mefloquine dose (mg/kg body weight)	
	15	25	15	25
No. of patients	56	53	53	50
% of patients who were:				
RI	23	20	35	33
RII	11	19	11	19
RIII	16	4	16	4
Sensitive	50	57	38	44

serum mefloquine concentrations (than patients with RI or sensitive responses (mean: 2023 µg/l versus 2666 µg/l;  $P = 0.09$ ).

## Discussion

Recent studies conducted along the Thailand–Myanmar (10) and Thailand–Cambodia (5) borders have shown that treatment of *P. falciparum* infections with mefloquine dosages of 15 mg/kg are associated with disturbingly high failure rates. Our results confirm these findings, with a failure rate of 62% on day 42 for the group that received 15 mg/kg—very similar to the 59% failure rate that we have previously reported for this area among patients who received this dose of mefloquine (5). We cannot exclude the reinfection of some patients, but the early treatment failures, i.e., the RII and RIII responses (which cannot be reinfections), accounted for a large proportion of all failures.

A single dose of 25 mg/kg of mefloquine did not improve the outcome: the 56% failure rate observed is close to the 62% found with the 15 mg/kg dose. This may be partly explained by the low mefloquine susceptibility of *P. falciparum* strains in this area: 13 patients with serum mefloquine concentrations >2000 µg/l on day 2, which used to be associated with successful treatment (6), exhibited an R response during the follow-up. With such highly mefloquine-resistant *P. falciparum* strains, an increase in the treatment dose was insufficient to achieve *in vivo* parasitocidal drug concentrations. In addition, mefloquine absorption seems to be erratic in adults at a single dose of 25 mg/kg, as shown by the large variation in the serum concentrations on day 2. The results of a similar study that compared 15 mg/kg and 25 mg/kg of mefloquine for the treatment of uncomplicated falciparum malaria on the Thailand–Myanmar border, where drug resistance to mefloquine has previously been documented (10), have recently appeared (11). In contrast with our findings, the higher dose of mefloquine was accompanied by a reduced risk of treatment failure: 40% of the patients treated with 15 mg/kg experienced a treatment failure by day 28, compared with only 9% of those treated with 25 mg/kg. It should be noted that the 25 mg/kg treatment was given in two doses separated by a 12–24-hour interval. These results are encouraging, but may have arisen because of the lower level of drug resistance of the *P. falciparum* strains on the Thailand–Myanmar border: the risk of treatment failure on days 7–9 was only 7% and 1% for the 15 mg/kg and 25 mg/kg regimen, respectively, compared with 27% and 23%, respectively, in our study.

Mefloquine consists of a racemic mixture of the (SR)-(–) and (RS)-(+) enantiomers. Both enantiomers

have the same effect against *P. bergii* in mice (12), and against a chloroquine-resistant *P. falciparum* strain *in vitro* (13). The (RS)-(+) enantiomer may be of particular interest since its biological half-life seems to be shorter (14) and its acetylcholinesterase and butyrylcholinesterase inhibitor activity may be lower (15). One consequence of its shorter half-life is that the selection pressure for drug-resistant strains of the malaria parasite could be lower in users of the pure (RS)-(+) enantiomer. The reduced esterase inhibitor activity might also lead to a lower frequency of adverse drug reactions among these users compared with users of the racemic mixture. The serum concentrations of the (RS)-(+) enantiomer were lower than those of the (SR)-(–) enantiomer in our study, a finding consistent with the results reported by Eggelte et al. (14) and Gimenez et al. (16). The very strong correlation between the serum enantiomer concentration and the total serum mefloquine concentration precluded differentiation between adverse reactions specific to either enantiomer and those associated with total mefloquine.

Adverse effects were more frequent with the 25 mg/kg single dose of mefloquine, but did not significantly differ from what was observed with the 15 mg/kg dose. The correlation between early vomiting within an hour of receiving mefloquine and a reduction in the concentration of mefloquine on day 2 is similar to the findings reported by Karbwang et al. (17). This underlines the need to re-treat patients who exhibit early vomiting. Patients with late vomiting should, however, not be re-treated since this might be due to high (“toxic”) concentrations of mefloquine, as demonstrated for Tanzanian children (18). It is of interest to note that the occurrence of dizziness on day 2 was strongly associated with higher serum concentrations of mefloquine.

Re-treatment with quinine–tetracycline was successful in 93% of instances.

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## Résumé

### Paludisme à falciparum dans l'est de la Thaïlande: essai randomisé sur l'efficacité d'une dose unique de méfloquine

Cet article présente les résultats d'un essai randomisé portant sur l'administration d'une dose unique de méfloquine (15 mg/kg ou 25 mg/kg de poids corporel) pour le traitement du paludisme à falciparum polypharmacorésistant non compliqué.

Au total, 110 sujets adultes ont été recrutés dans l'étude et répartis par tirage au sort dans des groupes recevant 15 mg/kg de méfloquine (57 sujets) ou 25 mg/kg (53 sujets). Les données de référence des patients ne différaient pas sensiblement entre les deux groupes, si l'on excepte une hémoglobémie plus faible dans le groupe à 15 mg/kg. Dans ce dernier groupe, 4 patients (7%) ont vomi dans l'heure suivant le traitement, 11 (19%) ont signalé avoir eu une diarrhée au cours des deux jours suivants, et 40 (70%) ont souffert de vertiges le jour 2. Dans le groupe à 25 mg/kg, les nombres correspondants de patients étaient 7 (13%), 14 (26%) et 39 (74%). Aucune de ces différences n'était statistiquement significative.

Au total, 7 patients (6%) (4 dans le groupe à 15 mg/kg et 3 dans le groupe à 25 mg/kg) n'ont pu être suivis pendant les 42 jours de l'étude. Les taux d'échec parasitologique dans les groupes à 15 mg/kg et 25 mg/kg étaient respectivement de 50% (28/56) et 43% (23/53) le jour 28, et 62% (33/53) et 56% (28/50) le jour 42. Il n'y avait pas de corrélation entre les échecs du traitement et les taux sériques de méfloquine le jour 2, puisque 13 sujets, sur les 19 ayant un taux de méfloquine sérique supérieur à 2000 µg/l le jour 2 ont présenté une réponse de type R au cours de la période de suivi. Le rapport moyen entre les concentrations des énantiomères (*SR*)-(-) et (*RS*)-(+) de méfloquine le jour 2 était de 3,37, ce qui montre qu'il existe des différences de pharmacocinétique entre ces deux formes.

Chez les sujets ayant présenté une réponse de type R, un nouveau traitement de sept jours par la quinine (30 mg/kg par jour) plus tétracycline (25 mg/kg par jour) a conduit à la guérison dans 93% des cas.

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