

DECLINING MEFLOQUINE SENSITIVITY OF *PLASMODIUM FALCIPARUM* ALONG THE THAI-MYANMAR BORDER

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Abstract. Mefloquine sensitivity of *Plasmodium falciparum* along the Thai-Myanmar border, both *in vitro* and *in vivo*, following different first-line treatments for uncomplicated falciparum malaria patients in these areas during the period 1997-2003 were studied. Standard *in vitro* micro tests and *in vivo* efficacy according to World Health Organization methodologies were performed. *P. falciparum* isolates along the Thai-Myanmar border with *in vitro* sensitivity to mefloquine have had up to a ten-fold decrease in sensitivity compared to a baseline done in 1986, conducted one year after the drug was first introduced to Thailand. The reduction in the mefloquine sensitivity of *P. falciparum* isolates in Tak Province developed rapidly, with the highest IC₅₀ of 1,254 nM in 1997. The IC₅₀ declined to 1,067 and 737 nM in 1999 and 2001, respectively, but there was no statistically significant difference in the sensitivity. The sensitivity of *P. falciparum* isolates from Mae Hong Son, Kanchanaburi, and Ranong, where the first line treatment was mefloquine 15 mg/kg single dose, continued to decline, where in 2001 the IC₅₀ were 1,087, 941, and 1,116 nM, respectively, in these provinces. The difference in sensitivities of *P. falciparum* isolates in Mae Hong Son and Ranong in 2001, compared to 1997, was statistically significant (p<0.05). Good therapeutic efficacy of the artesunate-mefloquine combination in Tak Province was observed. Adequate clinical responses (ACR) were 89.5% and 92.3% in 1997 and 2002, respectively. The efficacy of mefloquine alone in Mae Hong Son, Kanchanaburi, and Ranong has significantly dropped. ACR in 1997 and 2001 in Mae Hong Son were 87.8% and 73.2%, respectively, in Kanchanaburi were 82% and 59.6%, respectively, and in Ranong were 96% and 31.6%, respectively.

INTRODUCTION

Approximately 80% of malaria cases in Thailand occur along the country's international borders, with the highest proportion, about 70%, along the Thai-Myanmar border. Multidrug resistant *P. falciparum* is also a major problem in this area. This was first recognized along the Thai-Cambodian border. In the late 1980s and early 1990s, migration of gem miners from the Thai-Cambodian border to the Thai-Myanmar border resulted

in a rapid loss of mefloquine efficacy on both borders (Wernsdorfer *et al*, 1994; Thimasarn *et al*, 1995). The sensitivity to mefloquine of *Plasmodium falciparum* along the Thai-Myanmar border varied with the highest resistance found in Tak Province (Thimasarn *et al*, 1995; Wongsrichanalai *et al*, 2000). In 1995, treatment failure rates following mefloquine monotherapy for uncomplicated falciparum malaria were only 50% (Price *et al*, 1997). The Tak Province has the highest incidence of malaria, approximately one third of the country incidence (Malaria Section, 2003). The National Malaria Control Program (NMCP) of Thailand decided to change the first line treatment of uncomplicated falciparum malaria patients to a combination therapy of artesunate plus mefloquine in the Tak Province since 1995. In

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other provinces along the Thai-Myanmar border, where mefloquine cure rates are more than 70%, a single dose of 15 mg/kg mefloquine is still used as first line treatment. These include Mae Hong Son, Kanchanaburi, and Ranong Provinces. The response of falciparum malaria, both *in vitro* and *in vivo*, to standard treatments in these four provinces has been regularly monitored. The aim of this study was to analyze the sensitivities of *P. falciparum* along the Thai-Myanmar border, both *in vitro* and *in vivo*, to mefloquine antimalarials, and to compare those with the sensitivities of parasites in other areas where different first-line treatment are used.

MATERIALS AND METHODS

Study areas

This is a retrospective study focused on the mefloquine sensitivity of *P. falciparum* isolates along the Thai-Myanmar border. The *in vivo* and *in vitro* mefloquine sensitivity data conducted by the Antimalarial Drug Resistant Surveillance Program during the years 1997-2003 were analyzed. Four sentinel sites were selected. They were Mae Hong Son Province, along the northern border, Tak, along the northwestern border, Kanchanaburi, in the west, and Ranong, along the southern border (Fig 1).

Patients

All patients attending the malaria clinics in the sentinel sites were asked to participate in the study if they fulfilled the following criteria: proven mono-infection with *P. falciparum* malaria, asexual parasite density between 1,000 and 100,000/ μ l of blood, no intake of antimalarials in the preceding month, informed consent, and willingness to give blood samples. Ethical clearance for this study was obtained from the Ethical Review Committee of the Ministry of Public Health, Thailand.

In vitro test procedure

In vitro tests for the measurement of the drug sensitivity of *P. falciparum* followed the standard methodology for the assessment of inhibition of schizont maturation (WHO, 1990). Heparinized capillary tubes were used to collect 100 μ l of blood from each patient before treatment. Blood



Fig 1—Map of Thailand showing the study areas of Mae Hong Son, Tak, Kanchanaburi, and Ranong Provinces.

was drawn from finger after a prick with a sterile lancet and immediately placed in 900 μ l of RPMI 1640, pre-warmed to body temperature. A thick blood film was also prepared for reading pre-culture parasitemia. This was stained with 10% Giemsa at a pH of 7.2. WHO standardized mefloquine predosed plates was used. It was dosed with 0, 2, 4, 8, 16, 32, 64 and 128 pmol/well. Fifty microliters of the prepared blood-medium mixture was placed into each well of the plate, and incubated for up to 30 hours in a candle jar placed in an incubator, maintained at a temperature of 37.5°C (\pm 0.5°C). After incubation, parasites were harvested and Giemsa stained thick blood films were prepared and stained with 2% Giemsa at pH 6.8 for 30 minutes. The number of mature schizonts per 200 asexual forms of the parasites was used to assess maturation inhibi-

tion. Schizonts with at least 3 nuclei were defined as mature. Inhibitory concentrations (IC) and regression parameters were calculated using a computer adapted probit analysis of log-dose responses (Wernsdorfer and Wernsdorfer, 1995) based on the method of Litchfield and Wilcoxon (1949). Test of significance was based on potency ratio estimate for the comparison between 2 regression lines. If slope ratio (SR) was less than the factor of SR (fSR), the 2 lines were parallel within experimental error and the activities could be compared. If the potency ratio (PR) was greater than the factor of PR (fPR), the difference between the 2 lines was statistically different at $p < 0.05$.

***In vivo* test procedure**

The method used followed the WHO protocol for assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria (WHO, 1997; 2001). Uncomplicated falciparum malaria patients with an age over 6 months, 1,000-100,000 parasites/ μ l blood, an absence of general danger signs or signs of severe and complicated falciparum malaria, an axillary temperature $\geq 37.5^{\circ}\text{C}$, or a history of fever, an absence of febrile conditions caused by a disease other than malaria, the ability to comply with the stipulated follow-up visit and willingness to give informed consent were included in the study.

Subjects were asked to return to the malaria clinics for follow-up. Histories of symptoms or adverse drug reactions, including vomiting, rash, pruritis, and neuropsychiatric symptoms, were obtained on days 1, 2, 3, 7, 14, 21, and 28. Temperatures were measured and thick blood smears were prepared at the same time. Patients who failed to return were followed-up in their homes.

Treatment regimens

According to the national antimalarial drug policy guideline in 1995, treatment regimens for uncomplicated falciparum malaria were given based on the level of mefloquine resistance in each area. The Tak Province was classified as a high mefloquine resistant area; first line treatment was a combination of 25 mg/kg mefloquine plus 12 mg/kg artesunate and primaquine 30 mg in divided doses for two days. Mae Hong Son, Kanchanaburi, and Ranong were classified as low

mefloquine resistant areas; first line treatment regimen was a single dose of 15 mg/kg mefloquine plus primaquine 30 mg.

Outcome measures

The subject response to therapy was classified as follows. An early treatment failure (ETF) was defined as the development of signs of severe malaria with parasitemia on days 1, 2 or 3; a day 2 parasite density $\geq 100\%$ of day 0; parasitemia on day 3 with an axillary temperature $\geq 37.5^{\circ}\text{C}$; or a day 3 parasite density $\geq 25\%$ of day 0. A late treatment failure (LTF) was defined as the development of danger signs or severe malaria with parasitemia after day 3 or a clinical deterioration in the presence of parasitemia, or the reappearance of parasitemia between days 7 and 28 (same species as on day 0). An adequate clinical response (ACR) included subjects who did not fulfill the criteria of ETF or LTF, with negative blood smears on days 7, 14, 21, and 28.

RESULTS

***In vitro* sensitivity**

P. falciparum isolates along the Thai-Myanmar border have had a rapid decline in the *in vitro* sensitivity to mefloquine, with up to a ten-fold decrease, compared to the baseline sensitivity of 1986, conducted one year after the drug was first introduced to Thailand. Inhibitory concentration 50 (IC_{50}) is defined as a drug concentration that can inhibit 50% of schizont maturation. The geometric mean IC_{50} of mefloquine in 1986 ranged from 106 to 175 nM. The values increased to 737-1,116 nM in 2001 (Table 1). Reduction in the sensitivity of *P. falciparum* isolates in the Tak Province developed rapidly, with the highest IC_{50} of 1,254 nM in 1997. Other measures of IC_{50} declined to 1,067 and 737 nM in 1999 and 2001, respectively, but there is no statistical significance between these two years (Table 3). The sensitivity of isolates to mefloquine from Mae Hong Son, Kanchanaburi, and Ranong has continued to decline. The IC_{50} of the isolates from Mae Hong Son were 126, 539, 857, and 1,087 in the years 1986, 1997, 1999, and 2001, respectively. Those for the isolates from Kanchanaburi were 106, 624, 649, and 941, respectively. The IC_{50} values for the isolates from Ranong were 483,

Table 1
Geometric mean IC₅₀ of *P. falciparum* isolates from the Thai-Myanmar border in Tak, Mae Hong Son, Kanchanaburi, and Ranong Provinces to mefloquine antimalarial drugs in the years 1986, 1997, 1999 and 2001.

Year	Geometric mean of IC ₅₀ (n and 95% confidence intervals) in the Provinces			
	Tak	Mae Hong Son	Kanchanaburi	Ranong
1986	175 (32)	126 (32)	106 (17)	-
1997	1,254 (20; 887-1,772)	539 (28; 403-722)	624 (19; 467-834)	483 (26; 360-647)
1999	1,067 (61; 834-1,364)	857 (36; 681-1,078)	649 (31; 499-842)	783 (35; 581-1,056)
2001	737 (9; 412-1,319)	1,087 (15; 737-1,603)	941 (14; 572-1,547)	1,116 (29; 817-1,527)

Inhibitory concentration 50 (IC₅₀) values are the concentration of mefloquine that can inhibit 50% schizont maturation in *P. falciparum* isolates and is expressed in nmol/l blood.

Table 2
Comparison of log-probit regression lines representing the sensitivity of isolates from different places and years.

Regression lines	Parameter estimation				
	Slope ratio (SR)	Factor of SR (fSR)	Potency ratio (PR)	Factor of PR (fPR)	p-value
TK 2001 vs TK 1997	1.1319	1.9433	1.7015	2.823	p>0.05
MHS 2001 vs MHS 1997	1.166	1.5107	2.0167	1.6257	p<0.05 ^a
KB 2001 vs KB 1997	2.0115	1.7988	1.508	1.7780	p>0.05
RN 2001 vs RN 1997	1.5736	1.4971	2.3174	1.5354	p<0.05 ^a
TK 2001 vs MHS 2001	1.161	2.0118	1.4749	2.8659	p>0.05
TK 2001 vs KB 2001	1.2877	2.261	1.1498	2.9971	p>0.05
TK 2001 vs RN 2001	1.1344	1.9757	1.5156	2.7936	p>0.05
RN 2001 vs MHS 2001	1.317	1.597	1.0276	1.6471	p>0.05
RN 2001 vs KB 2001	1.1351	1.8764	1.7426	1.7996	p>0.05
MHS 2001 vs KB 2001	1.495	1.9134	1.6958	1.8799	p>0.05

MHS = Mae Hong Son Province; TK = Tak Province; KB = Kanchanaburi Province; RN = Ranong Province

^ap < 0.05 means statistically significant difference at a 5% confidence level

783, and 1,116 nM in 1997, 1999, and 2001, respectively. Statistically significant differences between the isolates collected in Mae Hong Son and Ranong in 1997 and 2001 were found (Table 2).

The *in vitro* sensitivities of *P. falciparum* isolates from Mae Hong Son, Kanchanaburi and Ranong, where first line treatment was mefloquine, single oral dose at 15 mg/kg mefloquine were quite similar in 2001. The geometric mean IC₅₀ values were 1,087, 941, and 1,116 nM, in these provinces, respectively (Table 1). There were no statistically significant differences between the isolates from these areas (p>0.05; Table 2). In the Tak Province,

where first line treatment was a combination of 25 mg/kg mefloquine plus 12 mg/kg artesunate in divided doses for two days, the isolates were more sensitive than those in Mae Hong Son, Kanchanaburi, and Ranong in 2001. The geometric mean IC₅₀ value was 737 nM, although there were no statistically significant difference between the isolates from Ranong, Kanchanaburi, and Mae Hong Son (p>0.05; Table 2).

Fig 2 shows a log-probit graph comparing dose-response regression lines representing *P. falciparum* isolates from the Tak, Mae Hong Son, Kanchanaburi, and Ranong Provinces. The line

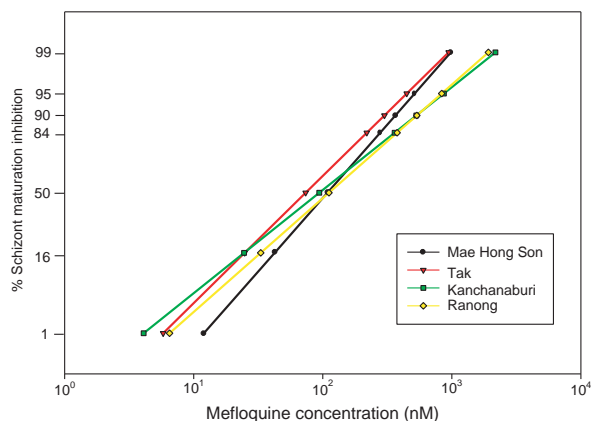


Fig 2—A log-probit graph comparing dose-response regression lines representing Tak, Mae Hong Son, Kanchanaburi, and Ranong isolates.

located to the left, closer to the Y axis, represents isolates with a lower IC_{50} than those to the right, meaning they are more sensitive isolates. The figure shows that the Tak isolates are more sensitive than the Mae Hong Son, Ranong, and Kanchanaburi isolates.

In vivo sensitivity

Good therapeutic efficacy for the ARS-MQ combination in the Tak Province was observed. Adequate clinical responses (ACR) were 89.5 and 92.3% in 1997 and 2002 respectively (Table 3). The efficacy of mefloquine alone in Mae Hong Son, Kanchanaburi, and Ranong has significantly dropped. ACR were 87.8% and 73.2% in Mae Hong Son, 82% and 59.6% in Kanchanaburi, and 96% and 31.6% in Ranong in 1997 and 2002, respectively (Table 3).

DISCUSSION

Mefloquine has been used for the treatment of uncomplicated falciparum malaria in Thailand since 1985 because it is effective and relatively well tolerated. Compliance is good because the drug can be given as a single dose. However, when used alone, it is likely to select resistant parasites because of its long half-life and slow elimination from the blood (Watkins and Mosobo, 1993).

Country surveillance data on antimalarial drug resistance has demonstrated a rapid decline in the mefloquine sensitivity of *P. falciparum* fol-

Table 3
Therapeutic efficacy of first line treatment regimens for uncomplicated falciparum malaria in Tak, Mae Hong Son, Kanchanaburi, and Ranong in 1997, 1999, and 2002.

Province	Year	n	ACR	ETF	LTF
Tak ^a	1997	38	89.5	-	10.5
	2002	39	92.3	2.6	5.4
Mae Hong Son ^b	1997	41	87.8	4.9	7.3
	2002	56	73.2	12.5	14.3
Kanchanaburi ^b	1997	50	82	6	12
	2002	109	59.6	22	18.3
Ranong ^b	1997	51	96	4	-
	2002	38	31.6	10.54	57.9

^aFirst line treatment regimen was MQ 25 mg/kg plus ARS 12 mg/kg in divided doses given for two days;

^bFirst line treatment regimen was MQ 15 mg/kg single dose; ACR = Adequate clinical response; ETF = Early treatment failure; LTF = Late treatment failure

lowing its introduction in 1985, especially along the Thai-Myanmar border. To reduce disease burden and to prevent or slow the emergence of mefloquine resistant *P. falciparum*, the NMCP decided to replace the mefloquine alone regimen with a combination of artesunate plus mefloquine. This first occurred in the Tak Province where the highest treatment failure rates (50% cure rate) were observed in 1995 (Thimasarn *et al*, 1995; Price *et al*, 1997). Following the change, successful treatment was obtained and sustainable. Cure rates for the artesunate- mefloquine combination were 89.5% and 92.3% in 1997 and 2002, respectively.

In addition, the *in vitro* sensitivity of *P. falciparum* clearly improved. In comparison, the situation in Mae Hong Son, Kanchanaburi, and Ranong, where mefloquine alone has been the first-line treatment regimen until 2002, *in vitro* assays of isolates collected from these areas demonstrate a progressive decline in mefloquine sensitivity from 1997 to 2001, supporting the high rate of treatment failure.

The replacement of mefloquine alone with the artesunate mefloquine combination in Kanchanaburi and Ranong occurred in late 2002, where the cure rate of mefloquine alone had

dropped to 59.6% and 31.6%, respectively. Current data, in 2003, (NMCP, in preparation for publication) indicate an improvement in treatment outcomes. Cure rates for the artesunate-mefloquine combination were 89.6% in Kanchanaburi and 94% in Ranong.

The improved *in vivo* efficacy of mefloquine following the change to the artesunate-mefloquine combination seen in this study supports the recommendation of the WHO (1998) for using combination therapy, especially with artemisinin derivatives, as a measure to improve treatment outcomes, and to delay the emergence of drug resistance, and reduce the prevalence of gametocyte carriage which can reduce transmission. Recovery in *in vitro* mefloquine sensitivity was observed among the isolates from Tak Province in 1999 and 2001.

An interesting finding was the mefloquine sensitivity of *P. falciparum* isolates in Tak Province described by the geometric mean IC_{50} . It shows the IC_{50} has declined gradually since the introduction of the artesunate-mefloquine combination in 1995. The 1997-2001 isolates from Mae Hong Son, Kanchanaburi, and Ranong, during which time mefloquine alone was used for the routine treatment of uncomplicated falciparum malaria, had a decline in the *in vitro* sensitivity to mefloquine, which is a significant finding observed in Mae Hong Son and Ranong (Table 1, 2).

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