# *IN VIVO* STUDY OF THE RESPONSE OF *PLASMODIUM FALCIPARUM* TO STANDARD MEFLOQUINE/SULFADOXINE/PYRIMETHAMINE (MSP) TREATMENT AMONG GEM MINERS RETURNING FROM CAMBODIA

Krongthong Thimasarn[1], Jeeraphat Sirichaisinthop[1] Saowanit Vijaykadga[1], Suwanna Tansophalaks[1], Phairoh Yamokgul[1], Aporn Laomiphol[1], Chatchai Palananth[1], Udom Thamewat[1], Sodsri Thaithong[2] and William Rooney[3]

[1]The Malaria Division, Department of Communicable Diseases Control, Ministry of Public Health, Bangkok; [2]Faculty of Science, Chulalongkorn University, Bangkok; [3]World Health Organization, Bangkok, Thailand

**Abstract.** An *in vivo* study of the response of *P. falciparum* to the combination drug, MSP, was conducted among gem miners who contracted malaria from Cambodia in 1991-1992. High level resistance (RII, RIII responses) was observed in 22.5% of the 40 cases attending Mae Sot malaria clinic, west Thailand border, and in 28.1% of the 96 cases attending Bo Rai malaria clinic, east Thailand border. The observations on *in vitro* studies conducted prior to the MSP treatment and after recrudescence, together with the findings on adequate mefloquine blood levels strongly indicated the serious deterioration of mefloquine efficacy. The first line treatment for the malaria control program needs to be revised and the use of qinghaosu derivatives considered. Intensive measures to combat spreading of the highly resistant strains to other parts of the country should be taken into account.

## INTRODUCTION

The treatment of falciparum malaria with the triple combination mefloquine-sulfadoxine-pyrimethamine (MSP or Fansimef), was introduced into Thailand early 1985. The drug was provided to falciparum malaria positives having a microscopic diagnosis, exceptions being pregnant women, small children, and sulfa-sensitive individuals. Evaluation of the efficacy of the regimen at the Thai-Cambodian border, conducted prior to introduction, indicated a 97% cure rate (Pinichpongse *et al*, 1987).

Monitoring of the sensitivity to mefloquine and quinine utilizing the standard WHO micro *in vitro* system was carried out on a longitudinal basis following base-line collection in 1982. Six teams operating from the 5 regions and headquarters of the Thai Malaria Control Program collected data from fixed representative monitoring stations on a yearly basis.

Results of the yearly monitoring indicated a reduction in sensitivity to the two drugs, quinine and mefloquine, during the period 1983-84. (Suebsaeng *et al*, 1986a) This may have been caused in part by excessive quinine pressure due to standard treatment for *P. falciparum* cases being quinine-tetracycline. These observations played a major role in the program decision to introduce MSP as an alternative drug. Following the introduction of MSP on an operational basis in 1985 decrease in the sensitivity to both quinine and mefloquine was noted (Suebsaeng, 1986 b).

In 1988-1991, Thailand experienced an epidemic of *P. falciparum* among gem miners returning from Cambodia. The epidemic took place in Bo Rai District, Trat Province, eastern Thailand, situated close to Pailin gem mines in Cambodia (Thimasarn, 1990a), Fig 1. These areas are well known as the epicenter of multidrug resistant falciparum strains.

These miners had arrived from various areas throughout the country for gem mining. It was estimated that 50% were from Trat and nearby provinces while 25% were from western border, namely Mae Sot, Tak Province (Thimasarn, 1990a).

*In vivo* studies conducted in Chanthaburi (the province nearby Trat) in 1989 demonstrated a marked reduction of cure rates from 97% in 1983-1984, 97.2% in 1985, 94.6% in 1987 to 55.2% in 1989. By the time the studies were conducted in Mae Sot, Tak, this area also demonstrated the similar findings *ie* 97.6% in 1985 to 53.1% in 1989 (Rooney *et al*, 1990; Karbwang *et al*, 1991). A separate evaluation by Medicins Sans Frontières and Mahidol University (Nosten *et al*, 1991) found a similar poor cure rate (71%) in Karen hilltribes contracting malaria in Mae Sot along the Myanmar border. Furthermore, routine

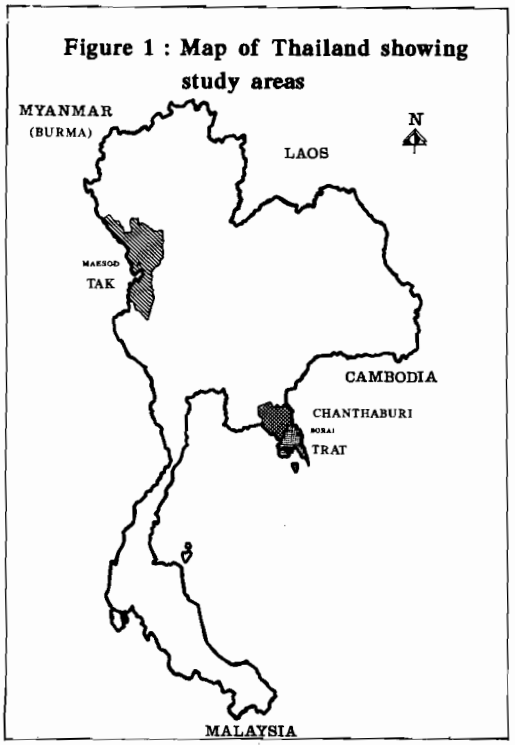


Fig 1–Map of Thailand showing study areas.

follow-up at the Bo Rai and Mae Sot clinics had indicated a high number of treatment failures.

Continuous culture *in vitro* conducted by Chulalongkorn University on 9 isolates collected from returning miners showed the MIC of all isolates to be higher than sensitive isolates and all were considered resistant *in vitro* to both mefloquine and quinine (Thaithong, 1989 personal communication). it is noteworthy that in this test system the same isolates showed sensitivity to chloroquine and amodiaquine.

It was unclear whether these cases in Mae Sot were contracted locally or whether had been contracted by Mae Sot gem miners while working in Cambodia (imported cases). Further it was assumed that the gem miners carrying resistant *Plasmodium falciparum* introduced these strains to the western highly endemic areas on returning to their place of origin. However, these *in vivo* studies were conducted at malaria clinics on an out-patient basis, and therefore reinfection which was usual in these high endemic areas and could be coincident factor, has to be ruled out. Furthermore the mefloquine blood levels should have been checked in order to distinguish inadequate levels from true resistance. A review of results obtained from the various sources, Malaria Division's *in vitro* monitoring, Chulalongkorn University's *in vitro* culture testing, together with the malaria clinic observations warranted an urgent investigation to determine clinical response of falciparum infections to MSP treatment among gem miners returning from Cambodia, thus necessitating possible alternate therapeutic regimens.

The objectives of this study were:

1) To confirm the presence of resistance to a standard MSP regimen in a controlled setting of symptomatic adult individuals with falciparum infections contracted while mining in Cambodia.

2) To describe the parasitologic pattern of resistance, *ie* relative proportions of S/RI, RII, RIII and patterns of parasitemias during the first 7 days.

## MATERIALS AND METHODS

The study was conducted in January 1991 - March 1992 and was divided in 2 parts as follows:

### 1) Controlled setting observations

This was conducted in Mae Sot malaria clinic, on symptomatic adult individuals, having contracted *P. falciparum* from Cambodia with parasitemia range 1,000 - 50,000 per µl blood and had no previous history of antimalarial drug intake over past 2 weeks prior to the study. The patients agreed to remain in malaria free area for the whole period of study *ie* 28 days to prevent reinfection. A bottle of repellent was distributed to each individual to assist in personal protection.

On admission each subject was administered, under supervision, 3 MSP tablets equal to 750 mg mefloquine, 1,500 mg sulfadoxine, 75 mg pyrimethamine. A single dose of primaquine (30 mg) was also administered, this being the usual regimen throughout the country. Thick blood smears collected daily until negative for 2 consecutive days then on days 7, 14, 21 and 28.

On recrudescence from day-7 onwards (or from any rising parasitemias requiring alternative therapy within the first 7 days) blood samples were collected

for continuous culture and *in vitro* testing at Chulalongkorn University, Bangkok. All recrudescent cases were retreated with standard doses of quinine plus tetracycline (7 days of each) with patients followed 7 days after retreatment for response.

**2) High level resistance determination**

The study aimed to determine possible presence of resistance at the RII and RIII levels. This was conducted in Bo Rai malaria clinic on gem miners returning from the Bolang/Khao Pet location in Cambodia (Approximately 30 km from Bo Rai).

The criteria to enrol patients was similar to those in controlled setting observations except that they were followed up daily for a period of one week after initial treatment, the reason being that this population group was highly mobile and would stay at the Bo Rai town for a short period of time before returning to Cambodia or their hometown.

On admission, the standard WHO micro *in vitro* tests were carried out on blood from selected subjects to determine response to mefloquine

Each subject was administered 3 MSP tablets equal to 750 mg mefloquine, 1,500 mg sulfadoxine, 75 mg pyrimethamine, plus a single dose (30 mg) primaquine.

Blood smears were collected on days 0, 1, 2, 3, 4, 5, 6 and 7. All RIII responses on day-2 (or significantly rising parasitemia before day-7) were administered alternative treatment with standard doses of quinine plus tetracycline for 7 days of each. All RII responses on day-7, as per WHO test interpretation for chloroquine, were observed for alternative treatment. Prior to re-treatment blood samples were collected for continuous culture and *in vitro* testing at Chulalongkorn University. Serum drug level estimations were conducted on day-3 on some sample cases.

The study design was approved by the Ethical Committee of the Ministry of Public Health Thailand.

**Statistical analysis**

Differences in proportions were analysed by chi square test and means by Students's *t*-test.

## RESULTS

Ninety-six and 40 patients were included in the studies in Bo Rai (eastern) and Mae Sot (western) malaria clinics, respectively. An average of 91% were adult males with mean age equal to 38.8 ± 16.8 years in Bo Rai group and 28.9 ± 11.1 years in Mae Sot group (Table 1).

Initial parasite densities prior to treatment were higher in Bo Rai group (13,558 ± 16,322 per µl) than in Mae Sot group (6,551 ± 9,680 per µl) but there was no statistical significant difference.

**Results of treatment**

The results of treatment are given in Table 2. The cure rate for the Mae Sot group was 36.1%. Nine of 40 cases (22.5%) showed high level resistance RII and RIII. The cure rate for Bo Rai group was not available since the study was a 7-day test. However,

Table 1

Patient biodata.

| Site | N | Age (yr) × ± 2SD | Female | Male | Initial parasite density (per µl) × ±SD* | Range |
|---|---|---|---|---|---|---|
| Bo Rai | 96 | 38.8 ± 16.8 | 12 (12.5%) | 84 (87.5%) | 13,558 ± 16,322 | 216 - 105,832 |
| Mae Sot | 40 | 28.9 ± 11.1 | 0 | 40 (100%) | 6,551 ± 9,680 | 136 - 48,464 |

*p = 0.107244

Table 2

Results of treatment.

| Site | No. of cases | Response S | S/RI | RI | RII | RIII | Cure rate on day-28 (%) |
|---|---|---|---|---|---|---|---|
| Bo Rai | 96 | - | 69 | - | 6 | 21 (28.1%) | NA |
| Mae Sot | 40 | 13 | 4 | 14 | 5 | 4 (22.5%) | 36.1 |

$$\text{Cure rate} = \frac{S}{N - S/RI} \times 100$$

Remarks : Evaluation of treatment results
S = Blood slides were negative for asexual forms from day-7 to day-28
RI = Initial disappearance of parasitemia, followed by recrudescence within 28 days
RII = Parasitemia reduction (asexual parasitemia of *less than* 25% of pretreatment count on day-2) but did not disappear within 7 days
RIII = No significant reduction (asexual parasitemia of equal or *more than* 25% of pretreatment count on day-2) of parasitemia within 7 days
S/RI = Blood slides were negative on day 7 and patients dropped out from the study before day-28

6 cases of RII response plus 21 cases of RIII response make 28.1% of total studied cases. This finding excludes possible reinfection.

**Vomiting**

Some mild side effects were observed among the two groups. Vomiting within 2 hours after drug intake was observed in 22.9% of Bo Rai group and 27.5% of Mae Sot group. Vomiting mostly occurred within the first hour after drug intake (82% in Bo Rai group and 91% in Mae Sot group).

There was a statistical correlation between vomiting and early treatment failure (RII + RIII) in the Bo Rai group but not in the Mae Sot group (Table 3, 4).

**Parasite clearance**

Among those cases with parasite clearance (*ie* excluding RII, RIII responses), 58% of the Bo Rai

Table 3

Vomiting within 2 hours and results of treatment in the Bo Rai group.

| Vomiting | Results of treatment (No. of cases) S/RI | RII + RIII | Total |
|---|---|---|---|
| No | 57 | 17 | 74 |
| Yes | 12 | 10 | 22 |
| Total | 69 | 27 | 96 |

$X^2 = 4.240002$, df = 1
p value $\leq 0.05$

Table 4

Vomiting within 2 hours and results of treatment in the Mae Sot group.

| Vomiting | Results of treatment (No. of cases) S + RI | RII + RIII | Total |
|---|---|---|---|
| No | 7 | 4 | 11 |
| Yes | 24 | 5 | 29 |
| Total | 31 | 9 | 40 |

$X^2 = 1.672340$, df = 1
p value > 0.1

group and 77% of the Mae Sot group showed parasite clearance within 3 days after treatment (Table 5). Pattern of daily parasite densities of initial clearance cases (N = 69) in the Bo Rai group is shown in Fig 2.

**Recrudescence**

Fourteen of 40 cases in the Mae Sot group showed RI responses. 57% of RI cases were observed during the third week after treatment (Table 6). This information was not available in the Bo Rai group since it was a 7-day test.

***In vitro* test on day 0**

*In vitro* tests using the WHO standard micro test technique were conducted among 50 eligible cases of the Bo Rai group prior to treatment. The responses expressed in EC/50, EC/90, EC/99 were 0.4263, 2.2285 and $8.5811 \times 10^{-6}$ mol/l, respectively. These effective concentrations (EC) were much higher than those in tests conducted before the introduction of this drug to the area (1983-1984) (Table 7). These findings also showed a correlation between *in vitro* and *in vivo* responses of mefloquine resistant cases.

***In vitro* test on recrudescence**

Some parasitized blood isolates were collected from both groups prior to retreatment with quinine plus tetracycline. Unfortunately, due to low parasitemias and long distance transportation, only 5 isolates from the Bo Rai group and 14 isolates from the Mae Sot group were successfully established in continuous culture.

*In vitro* data shown in Table 8 confirmed marked resistance to both mefloquine and quinine.

Table 5

Day of parasite clearance.

| Site | Total cases | No. of cases with parasite clearance * | Day of parasite clearance | | Percentage of parasite clearance within 3 days |
|---|---|---|---|---|---|
| | | | Day 2 | Day 3 | |
| Bo Rai | 96 | 69 | 15 (21.7%) | 25 (36.2%) | 58 |
| Mae Sot | 40 | 31 | 14 (45.2%) | 10 (32.2%) | 77 |

Note * *ie* All cases excluding RII and RIII responses

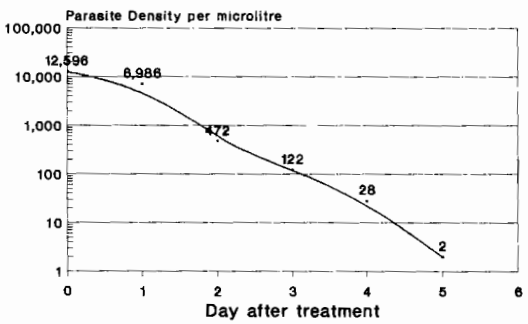


Fig 2–Mean parasite density of Bo Rai patients with parasite clearance after initial treatment with MSP.

**Mefloquine blood levels**

A total of 80 blood samples from Bo Rai were collected on day-3 after treatment. Mefloquine levels were measured in whole blood by high performance liquid chromatography. The detail of analysis have been published elsewhere (Karbwang *et al*, 1993). Mefloquine concentrations in patients in the S/RI group were significantly higher than those with early treatment failure (RII, RIII) with the respectively (SD) values of 1959 (696) and 1622 (863) ng/ml.

The mefloquine concentrations in patients with RII, RIII responses were higher than those with a sen-

Table 6
Day of recrudescence.

| Site | 0-7 RII, RIII | 8-14 ← | 15-21 RI | 22-28 → | Total | Note |
|---|---|---|---|---|---|---|
| Bo Rai | 27 | NA | NA | NA | 27 | 7-day test |
| Mae Sot | 9 | 3 (21%) | 8 (57%) | 3 (21%) | 23 | |

Table 7

*In vitro* response of *P. falciparum* to mefloquine in the standard WHO micro-test, Chanthaburi-Trat (Bo Rai) area, 1982-1992.

| Year | Site * | No. of tests | Results EC 50 | EC 90 ($\times 10^{-6}$ mol/l) | EC 99 |
|---|---|---|---|---|---|
| 1982 | C | 44 | 0.0920 | 0.4077 | 1.3736 |
| 1983 | C | 39 | 0.0979 | 0.2386 | 0.4937 |
| 1984 | C | 40 | 0.1171 | 0.4470 | 1.3330 |
| 1985 | C | 39 | 0.0971 | 0.2694 | 0.6192 |
| 1986 | C | 32 | 0.0723 | 0.3113 | 1.0246 |
| 1988 | C | 40 | 0.2408 | 0.8059 | 2.1573 |
| 1989 | C | 33 | 0.1890 | 0.8911 | 3.1541 |
| 1990 | T | 51 | 0.4311 | 1.4088 | 3.6991 |
| 1991-2 | T | 50 | 0.4263 | 2.2285 | 8.5811 |

* Remark C = Chanthaburi T = Trat (Bo Rai)
Source: 1982-1990 data from Malaria Division, Ministry of Public Health

sitive response in a previous study. These findings confirmed that true mefloquine resistant parasites did exist and ruled out the inadequate blood levels of mefloquine.

## DISCUSSION

The findings indicated true mefloquine resistance at the Thai-Cambodian border (Bo Rai). The rapid development of mefloquine resistance in this area is most likely due to the uncontrolled falciparum malaria epidemic among gem miners who crossed the border to Pailin in Cambodia. The epidemic commenced in late 1988 and ended in 1992. The mefloquine in this study was administered in combination with sulfadoxine/pyrimethamine, MSP. However the SP components would express limited additive effect on the parasites since the area is long known for high level sulfadoxine/pyrimethamine resistance and the evidence in a previous study showed that there was no significant difference between the single drug and the combination (Thimasarn *et al*, 1990a, b).

The drop in cure rates of MSP at the Thai-Myanmar border (Mae Sot) conducted by the drug sensitivity monitoring team, was parallel to those in the studies at the Thai-Cambodian border (Rooney *et al*, 1990; Karbwang *et al*, 1991). This strongly indicated that mefloquine resistant falciparum has been transferred in an east-west direction by parasites transported by the gem-miners.

Some recent studies conducted during the same period confirmed the presence of mefloquine re-

Table 8

*In vitro* response of *P. falciiparum* to mefloquine and quinine prior to retreatment of recrudescence.

| Isolates | Treatment results | Day of blood collection after initial treatment | *In-vitro* sensitivity (mol/l) | |
|---|---|---|---|---|
| | | | MEF | QNN |
| BR 24 | R III | 5 | $2\text{-}5 \times 10^{-7}$ | $5 \times 10^{-7}$ |
| BR 27 | R III | 6 | $2\text{-}5 \times 10^{-7}$ | $3\text{-}5 \times 10^{-7}$ |
| BR 79 | R III | 7 | $2\text{-}5 \times 10^{-7}$ | $5 \times 10^{-7}\text{-}10^{-6}$ |
| BR 93 | R II | 7 | $1\text{-}5 \times 10^{-7}$ | $3\text{-}5 \times 10^{-7}$ |
| BR 94 | R III | 7 | $2 \times 10^{-7}$ | $1\text{-}3 \times 10^{-7}$ |
| MS 1 | R II | 7 | $5 \times 10^{-7}$ | $5 \times 10^{-7}\text{-}10^{-6}$ |
| MS 2 | R I | 28 | $2\text{-}5 \times 10^{-7}$ | $2\text{-}5 \times 10^{-7}$ |
| MS 4 | R I | 28 | $5 \times 10^{-7}$ | $2\text{-}5 \times 10^{-7}$ |
| MS 10 | R II | 7 | $5 \times 10^{-7}$ | $2 \times 10^{-7}\text{-}10^{-6}$ |
| MS 14 | R I | 21 | $5 \times 10^{-7}$ | $2\text{-}5 \times 10^{-7}$ |
| MS 15 | R II | 7 | $5 \times 10^{-7}$ | $1\text{-}5 \times 10^{-7}$ |
| MS 20 | R II | 14 | $5 \times 10^{-7}$ | $5 \times 10^{-7}$ |
| MS 21 | R I | 21 | $2\text{-}5 \times 10^{-7}$ | $2\text{-}5 \times 10^{-7}$ |
| MS 23 | R III | 14 | $5 \times 10^{-7}$ | $2 \times 10^{-7}$ |
| MS 24 | R I | 21 | $5 \times 10^{-7}$ | $5 \times 10^{-7}$ |
| MS 27 | R I | 21 | $5 \times 10^{-7}$ | $5 \times 10^{-7}$ |
| MS 33 | R III | 7 | $2\text{-}5 \times 10^{-7}$ | $2\text{-}5 \times 10^{-7}$ |
| MS 36 | R I | 28 | $5 \times 10^{-7}$ | $2 \times 10^{-7}$ |
| MS 41 | R I | 14 | $5 \times 10^{-7}$ | $5 \times 10^{-7}$ |
| Cut point for resistance | | | $10^{-7}$ | $5 \times 10^{-7}$ |

sistance, both *in vivo* and *in vitro*. Mefloquine alone (Lariam®), 15 mg/kg dose which is equivalent to 750 mg in adults, cured only 40% of cases during 1991 at a Cambodian refugee camp 100 kilometers north of Bo Rai (Fontanet *et al*, 1993). This study also reported 53% high degree resistance (RII, RIII). Another study conducted in an area 20 kilometers north of Bo Rai showed that mefloquine 750 mg single dose gave 33.3% cure rate during 1990-1991 (Ketrangsee *et al*, 1992). The study conducted by Médicins Sans Frontières on the northern-Thai-Cambodian border in 1991-1992 showed 40% failure of mefloquine 15 mg/kg, whereas mefloquine 25 mg/kg showed 11% failure (Smithuis *et al*, 1993).

*In vitro* findings conducted by another team at Bo Rai in 1990 also showed a similar pattern as found in our study (Wongsrichanalai *et al*, 1992).

On the opposite border (Thai-Myanmar), the study carrried out at a Karen refugee camp 110 km north of Mae Sot showed that MSP at a single dose of 15/30/1.5 mg/kg respectively, cured only 71% of cases. This study also reported 6% early treatment failures (RII, RIII) and demonstrated that inadequate mefloquine concentrations were not responsible for the high treatment-failure rate (Nosten *et al*, 1991). At the same area in 1990, mefloquine alone 15 mg/kg cured 60% of cases and an increased dosage to 25 mg/kg was recommended (ter Kuile *et al*, 1992).

The routine *in vivo* studies conducted continuously at Mae Sot malaria clinic by the Malaria Division showed that the cure rates of MSP among locally contracted cases dropped rapidly. During March-May 1990, 65% of patients attending Mae Sot clinic contracted infection from Cambodia, whereas, only

26% contracted infections locally (Sirichaisinthop, 1993 personal communication). This suggests that massive population migration between Bo Rai and Mae Sot plays a major role in the spread of mefloquine resistant strains.

The controversial findings on vomiting among the 2 groups indicated that vomiting should not relate with early treatment failure. This is confirmed by the adequate mefloquine blood levels in Bo Rai patients.

We observed a marked delay in parasite clearance among Bo Rai patients with S/RI responses when compare with those observed before the epidemic. Unfortunately the baseline data conducted in 1983-1984 at the same area did not document statistics on parasite clearance time (Pinichpongse *et al*, 1987). However, many previous clinical studies reported an approximate 48 hours parasite clearance time. Upon review of the individual daily parasite density of Bo Rai patients we observed that there were a fluctuation of parasite densities, this might be due to multi-strains. Moreover, some cases that were classified as RII or RIII according to WHO criteria while having low parasite densities and showed no deterioration of clinical signs, were not retreated with the second line drug immediately. This suggests that the WHO criteria for evaluation of treatment results using chloroquine model may not be suitable for longer acting drugs such as mefloquine.

The serious problem on mefloquine resistance and the apparent spreading of resistant strains to the western border and to other parts of the country necessitates urgent control measures that includes revision of the first line treatment policy, vector control, and other means of drug resistant containment. Qinghaosu and its derivatives need to be carefully considered as the second line drugs. The 7 day-quinine and tetracycline seems to be unsatisfactory due to poor patient compliance together with the quinine-mefloquine like chemical structure.

## ACKNOWLEDGEMENTS

This work was kindly funded by the Malaria Unit, World Health Organization, Geneva. We are grateful to staff of the Malaria Sector 4 Bo Rai and the Malaria Sector Mae Sot. We also thank Dr Surang Tanpradist, Dr Somsak Prachakwong, Regional Directors of Malaria Region I and V for their kind permission to conduct this study, and Ms Suranya Pundara for her assistance in typing the manuscript.

## REFERENCES

Fontanet AL, Johnson BD, Walker AM, *et al.* High prevalence of mefloquine resistant falciparum malaria in eastern Thailand. *Bull WHO* 1993; 71 : 377-83.

Karbwang J, Harinasuta T. Drug Resistant Malaria. *WHO Tech Report Ser* 1991; 1 : 40-65.

Karbwang J, Na Bangchang K, Thimasarn K, *et al.* Mefloquine levels in patients with mefloquine resistant *Plasmodium falciparum* in the eastern part of Thailand. *Southeast Asian J Trop Med Public Health* 1993; 24 : 226-9.

Ketrangsee S, Vijaykadga S, Yamokgul P, *et al.* Comparative trial on the response of *Plasmodium falciparum* to halofantrine and mefloquine in Trat Province, eastern thailand. *Southeast Asian J Trop Med Public Health* 1992; 23 : 55-8.

Nosten F, Kuile FT, Chongsuphajaisiddhi T, *et al.* Mefloquine-resistant falciparum malaria on the Thai-Burmese border. *Lancet* 1991; 337 : 1140-3.

Pinichpongse S, Suebsaeng L, Malikul S, *et al.* The operational introduction of mefloquine, a new antimalarial drug by the Malaria Program of Thailand. *Commun Dis J* 1987; 13 : 411-24.

Rooney W, Thimasarn K, Yamokgul P, *et al.* The response of *Plasmodium falciparum* to Mefloquine and Quinine in Thailand, Status Report July 1990 (unpublished).

Rooney W, Thimasarn K. Development of Multi-Drug Resistance in Forest Related Falciparum Malaria. Forest Malaria in Southeast Asia, Proceedings of an Informal Consultative Meeting WHO/MRC 18-22 February 1991, New Delhi : World Health Organization, 1991; 227-34.

Smithuis FM, van Woensel JBM, Nordlander E, *et al.* Comparison of two mefloquine regimens for treatment of *Plasmodium falciparum* malaria on the northeastern Thai-Combodian Border. *J Antimicrob Agents Chemother* 1993; 37 : 1977-81.

Suebsaeng L, Wernsdorfer WH, Rooney W. Sensitivity to quinine and mefloquine of *Plasmodium falciparum* in Thailand. *Bull WHO* 1986a; 64 : 759-65.

Suebsaeng L, Progress report on regional collaborative studies on drug resistant malaria, Thailand. 1986b; 1-14.

ter Kuile FO, Nosten F, Thieren M, *et al.* High dose mefloquine in the treatment of multidrug-resistant falciparum malaria. *J Infect Dis* 1992; 166 : 1393-400.

Thimasarn K. Malaria in Bo Rai. *Thai Malaria J* 1990a; 25 : 57-66.

Thimasarn K, Pinichpongse S, Malikul S, *et al.* Phase III double-blind comparative study of Fansimef® and Lariam® for the curative treatment of *Plasmodium falciparum* infections in Thailand. *Southeast Asian J Trop Med Public Health* 1990b; 21 : 404-11.

Wongsrichanalai C, Webster HK, Wimonwattrawatee T, *et al.* Emergence of multidrug resistant *Plasmodium falciparum* in Thailand. *In vitro* tracking. *Am J Trop Med Hyg* 1992; 47 : 112-6.