RESEARCH NOTE

EFFICACY OF MEFLOQUINE TREATMENT AND GENETIC PROFILES IN UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN SOUTHERN LAO PDR

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Abstract. We conducted a 28-day follow-up of 17 Laotian patients diagnosed with uncomplicated *Plasmodium falciparum* malaria treated with mefloquine (Mephaquine®, MQ) alone to determine the efficacy. All patients were completely cured with MQ, without reappearance of asexual stage parasitemia at follow-up. Of the 7 isolates tested for genotypic analysis, one isolate was a Y86 mutant type of the *pfmdr1* gene, the others were N86 wild. These findings suggest no MQ resistance in the study area possibly because the drug is rarely used in southern Lao PDR.

Keywords: *Plasmodium falciparum,* mefloquine, genetic profile, Lao PDR

INTRODUCTION

Malaria is a serious public health problem in Lao PDR, accounting for an estimated 1,561 deaths from 1998 to 2002, as recorded by the Center for Malariology, Parasitology and Entomology (CMPE), Vientiane. The threat of malaria is particularly serious in remote areas where there are frequent outbreaks and access to medical services is difficult. Although preventive measures such as the use of impregnated bednets and health education have played a role in reducing morbidity and mortality due to malaria in recent years, there is growing concern about the increasing frequency of treatment failure. An alarming increase in falciparum malaria resistant to chloroquine and sulfadoxine-pyrimethamine, two commonly used anti-malarial agents

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for treating uncomplicated malaria in Lao PDR, has been reported in several *in vivo* and *in vitro* studies from Lao PDR (Tawil, 1977; Gibota *et al*, 1992; Pillai *et al*, 2001; Mayxay *et al*, 2003). The national policy for treatment of uncomplicated falciparum malaria changed from chloroquine to artemisinin-based combination therapy (artemether/lumefantrine) as first line treatment in 2006.

Mefloquine (MQ), is currently recommended as a prophylactic agent but has been used to treat uncomplicated falciparum malaria in Southeast Asia since the 1980's as it is effective and relatively well tolerated. Since the first case of MQ resistance was reported in Thailand in 1982, *in vitro* resistance to MQ has been demonstrated. It has been reported from Thailand malaria cure rates using MQ are less than 50% (Fontanet *et al*, 1993; Price *et al*, 1997). Thus, there is concern about the future use of MQ. The problem of MQ-resistant malaria has also occurred in Myanmar, Cambodia, China and Vietnam (Jiang *et al*, 1982; Lapiere *et al*, 1983; Anh *et al*, 1990; Wongsrichanalai *et al*, 2001). MQ has not appeared on the market in Lao PDR because of reports of treatment failure with MQ in neighboring countries and the cost. There are no reported data regarding MQ resistance in Lao PDR. In view of reported multidrug-resistance in neighboring countries (Gomes *et al*, 1978; White, 1992; Smithuis *et al*, 1997), the importation of MQ resistant malaria from neighboring countries to Lao PDR is a real possibility, especially because of population migration across borders. Therefore, study of MQ resistant malaria is vital.

The present study assessed the efficacy of MQ for treating uncomplicated falciparum malaria in a selected region of Lao PDR.

MATERIALS AND METHODS

We carried out an *in vivo* treatment trial using MQ to treat uncomplicated cases of malaria in southern Lao PDR and conducted genotypic analysis of some of those cases to determine the mechanism of resistance.

This study received approval from Ministry of Health, Lao PDR to be conducted in August 2004 in close co-operation with senior officials of the CMPE, who helped in the selection of study sites, the field setting, language interpretation and technical expertise. This study was conducted during the rainy season in rural Phouvong District, Attapeu Province, Lao PDR; a malaria endemic area with moderate transmission located about 1,000 km south of Vientiane. The residents of the study areas belong to two ethnic groups: Lao Luom (lowland) and Lao Theung (upland), who live by agriculture and farming.

RESULTS

A total of 887 people were examined for *Plasmodium falciparum* by microscopy using thick and thin blood smears from finger prick blood (Table 1). Of these, 138 were positive for *P. falciparum.* Sixteen of the positive samples also had mixed infection with *P. vivax*. Twenty-five cases were positive for *P. vivax* only by microscopy using a Giemsa stained blood film. One case was identified to be *P. malariae*. The overall prevalences were *P. falciparum* in 13.8%, *P. vivax* in 2.8%, mixed infections in 1.8% and *P. malaria* in 0.1%, giving an overall prevalence of malaria of 18.5%.

Of the 138 *P. falciparum* positive cases, 17 (10 males and 7 females aged 2-10 years), after obtaining informed consent from the parents and meeting inclusion

Table 1
Results of examination for malaria infection in Phouvong District, Attapeu Province, 2004.

Village	Number of inhabitants (estimated)	Number of inhabitants examined (%)	F positive cases (%) with Paracheck	Microscopy (%)				
				F	V	M	FV	Total
Vongsamphan	1,369	132 (9.6)	6 (4.5)	7 (5.3)	0 (0)	0 (0)	0 (0)	7 (5.3)
Vongvilai	844	218 (25.8)	40 (18.3)	41 (18.8)	5 (2.3)	0 (0)	3 (1.4)	49 (22.5)
Vongngan	784	262 (33.4)	27 (10.3)	25 (9.5)	9 (3.4)	0 (0)	2 (0.8)	36 (13.7)
Ta-Oum	275	116 (42.2)	25 (21.6)	27 (23.3)	8 (6.9)	0 (0)	3 (2.6)	38 (32.8)
Phouhom	606	159 (26.2)	31 (19.5)	22 (23.8)	3 (1.9)	1 (0.6)	8 (5.0)	34 (21.4)
Total	3,878	887 (22.9)	129 (14.5)	122 (13.8)	25 (2.8)	1 (0.1)	16 (1.8)	164 (18.5)

F, *Plasmodium falciparum;* V, *P. vivax;* M, *P. malariae*

Table 2
Results of 28-day follow-up of using mefloquine against *Plasmodium falciparum* malaria in Attapeu Province, Lao PDR.

Features	Result
No. patients	17
Mean age (range)	6 (2-10) years
Percent male	58.8
Parasitemia (range)	5,427 (1,040-24,800)/ l
Axillary temperature (range)	37.5ºC (37.0-38.9ºC)
Parasitologic response	
Sensitive	17/17 (100%)
Resistant	0/17 (0%)

criteria were included in the study. The inclusion criteria were having signs and symptoms of acute uncomplicated *P. falciparum* malaria (fever less than 39.5ºC by axillary temperature at presentation or a history of fever within the previous 24 hours), a single infection with *P. falciparum*, and an initial parasitemia (asexual stage parasites) of > 1,000/microliter blood. The selected cases were administered MQ (25 mg/kg body weight) in divided doses for 3 days (Days 0, 1 and 2) and were followed up on Days 2, 3, 7, 14, 21 and 28 for axillary temperature, thick and thin blood smears and blood spots on filter paper. Giemsa's stained blood smears were read by reference microscopists of the CMPE and filter paper blood was used for analysis of *pfmdr1* polymorphisms of the *P. falciparum* isolates, which is considered to be associated with MQ resistance.

The results of the 28-day follow-up of the *in vivo* treatment trial with MQ are summarized in Table 2. After treatment, all patients in the *in vivo* trial were

Table 3
Lab results for 17 cases of *Plasmodium falciparum* treated with mefloquine.

No.	Sex	Age	Day of blood examination (parasite count/mm^3)							Result
			D0	D2	D3	D7	D14	D21	D28	
1	M	8	1,120	-	-	-	-	-	-	Sensitive
2	M	3	1,160	G	G	-	-	-	-	Sensitive
3	F	8	1,600	G	G	-	-	-	-	Sensitive
4	F	7	6,400	-	-	-	-	-	-	Sensitive
5	M	6	24,800	176	-	-	-	-	-	Sensitive
6	M	5	2,320	-	-	-	-	-	-	Sensitive
7	M	10	1,080	-	-	-	-	-	-	Sensitive
8	F	8	10,800	G	G	-	-	-	-	Sensitive
9	M	6	3,120	G	-	-	-	-	-	Sensitive
10	M	3	18,500	G	G	-	-	-	-	Sensitive
11	M	6	1,160	G	-	-	-	-	-	Sensitive
12	M	7	2,400	-	-	-	-	-	-	Sensitive
13	F	3	1,040	-	-	-	-	-	-	Sensitive
14	F	5	2,520	G	G	-	-	-	-	Sensitive
15	M	2	1,500	G	G	G	-	-	-	Sensitive
16	M	8	10,800	-	-	-	-	-	-	Sensitive
17	F	8	2,120	-	-	-	-	-	-	Sensitive

-, clearance; G, gametocytes only

completely cured. The peripheral blood was cleared of asexual parasites by Day 2 in 16 patients and by Day 3 in the remaining 1 patient. The gametocytes that remained in some patients after treatment disappeared within 14 days of treatment (Table 3).

Pfmdr1 polymorphisms were seen in 7 *P. falciparum* positive samples. At position 86, all isolates were wild type except one isolate, which was mutant N86Y. No polymorphisms were seen at position 1246. These results were consistent with those of the *in vivo* trial.

DISCUSSION

Considering the situation of the neighboring countries, it is unexpected the resistance to MQ was not seen *in vivo* in our test in southern Lao PDR. This would reflect the situation in which there is no drug pressure to cause MQ resistance because MQ is rarely used. There appears to have been no imported resistance by migrants from Cambodia, where MQ resistance by *P. falciparum* exists (Lim *et al*, 2005).

Lao PDR is in the process of changing its national malaria drug policy from chloroquine to artemisinin-based combination therapy. Although MQ is not used alone for clinical therapy in this country, it is available as a partner drug, for use in combination therapy with artemisinins or as a prophylactic agent in travelers.

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