AN OPEN, RANDOMIZED TRIAL OF THREE-DAY TREATMENT WITH ARTESUNATE COMBINED WITH A STANDARD DOSE OF MEFLOQUINE DIVIDED OVER EITHER TWO OR THREE DAYS, FOR ACUTE, UNCOMPLICATED *FALCIPARUM* MALARIA

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Abstract. The combination of artesunate and mefloquine is currently one of the most effective treatments for multidrug-resistant *Plasmodium falciparum* malaria. Simultaneous, rather than sequential treatment with the two drugs, would allow better patient compliance. We therefore evaluated three-day treatment with artesunate combined with either 2 or 3 days of mefloquine co-administered once a day with artesunate. The study was an open, randomized trial for acute, uncomplicated falciparum malaria and was conducted at the Bangkok Hospital for Tropical Diseases. One hundred and twenty adult patients were randomized to two treatment groups. Group 1 patients received 4 mg/kg/day of artesunate for 3 days and 3 daily doses of 8.0 mg/kg/day mefloquine given with artesunate. Group 2 patients received the same dose of artesunate and the same total dose of mefloquine (25 mg/kg). However, the mefloquine was given as 15 mg/kg on the first day and 10 mg/kg/ on the second day, again with artesunate. The baseline demographic and clinical characteristics of the patients in the two groups were similar. The cure rates for the 3-day and 2-day mefloquine regimens were 100% and 99%, respectively. There were no significant differences in either median fever clearance times (group 1=32 hours; group 2=33 hours) or mean parasite clearance times (group 1=42.3 hours; group 2=43.3 hours). Both regimens were well tolerated and there were no significant differences in the incidence of adverse effects. Nausea or vomiting occurred in 3.8% of patients in both groups and transient dizziness occurred in 4% of group 1 and 9% of group 2 patients. These results suggest that a 3-day regimen of mefloquine administered with artesunate is effective and well tolerated. This practical regimen could improve patient compliance.

INTRODUCTION

The incidence of multidrug-resistant *Plasmodium falciparum* malaria is increasing and is becoming a public health problem in tropical countries. Combination regimens of two antimalarial drugs with different targets of action have been shown to delay the development of drug resistance and to improve cure rates (Nosten *et al*, 2000). The most highly-resistant isolates of *P falciparum* are found in Southeast Asia (Looareesuwan *et al*, 1992a). Effective treatment for uncomplicated malaria due to these highly resistant strains relies on the combination of an artemisinin derivative, such as artesunate, with mefloquine (Looareesuwan *et al*, 1994, 1996; Nosten *et al*, 1994).

Artesunate is very well tolerated, but in order to be effective as monotherapy, treatment for at least 5 days is required (Looareesuwan *et al*, 1992b, 1997). When used alone, recrudescence rates in the order of 10% to 20% have been reported, which may be attributable to the short half life of the compound (Li *et al*, 1994; Baradell and Fitton, 1995). Mefloquine was introduced in the 1980s and remains effective in most endemic areas for both the treatment and prophylaxis of malaria. The drug is generally well tolerated but may occasionally be associated with neuropsychiatric adverse effects (White, 1994; Palma *et al*, 1993). A series of controlled studies showed high efficacy rates and good tolerability for the combination (Price *et al*, 1999;

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Luxemburger *et al*, 1994; Bunnag *et al*, 1996, 1997; Price *et al*, 1995, 1998; Thimasarn *et al*, 1997; van Vugt *et al*, 1998), and consequently, it has become a standard regimen for the treatment of uncomplicated malaria in Southeast Asia. However, treatment recommendations differ, in regards to both duration, from 1 day (Luxemburger *et al*, 1994) to 5 or more days (Looareesuwan *et al*, 1992b; Price *et al*, 1998), and in regards to dose. Mefloquine is generally begun at the end of the course of artesunate or used on the first and second days of artesunate treatment. However, the dose and duration of mefloquine administration remain controversial and combination therapy may present logistical problems and lead to decreased patient compliance. A fixed dose combination has the advantage of simplicity and allows the drugs to be combined in one formulation.

The aim of the present study was to investigate the efficacy and safety of simultaneously administered artesunate 4 mg/kg/day (total dose 12 mg/kg) given once daily for a total of 3 days with mefloquine (total dose 25 mg/kg) divided into three doses (8 mg/kg/day) given once daily. This regimen was compared to a conventional artesunate-mefloquine sequential combination regimen (artesunate 4 mg/kg/day given once daily for a total of 3 days together with mefloquine 15 mg/kg/day co-administration on the first day and 10 mg/kg/day on the second day).

MATERIALS AND METHODS

This study was conducted in Thailand at the Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. Study approval was given by the Ethics Committee of the Faculty of Tropical Medicine. Patients 15 years of age or older weighing at least 39 kg and presenting with microscopically confirmed *Plasmodium falciparum* malaria were eligible for the trial if they or a responsible family member gave informed consent. Subjects with signs and symptoms of severe or complicated malaria (World Health Organization, 2000) were excluded, as were individuals unable to tolerate oral medications, pregnant or lactating females, individuals allergic to study drugs or patients who had taken any antimalarial drug within 2 weeks of admission. All patients were admitted to the hospital for 28 days or agreed to stay in a non-malaria endemic area for 28 days to exclude re-infection. All patients were followed up weekly.

Each patient was sequentially assigned to one of two regimens: Group 1, patients were given artesunate 4 mg/kg/day for three days, (Gullin Pharmaceutical Corp, China, 50 mg/tablet) with mefloquine 8 mg/kg/day (250 mg/tablet) once a day for three days. Group 2, patients were given artesunate 4 mg/kg/day for three days with mefloquine 15 mg/kg/ on the first day and 10 mg/kg on the second day. Artesunate was given with mefloquine and patients who vomited within one hour after drug administration were redosed with both drugs.

On enrollment, a medical history was obtained, a full baseline physical examination was conducted, and blood was taken for routine hematology and biochemistry. Clinical signs and symptoms were recorded daily during the first week and then weekly until patients were discharged. Oral temperature, pulse, and respiratory rates were measured every 4 hours and the blood pressure was recorded once daily. Routine laboratory examinations were performed on days 0, 7, 14, 21, and 28. Malaria parasite counts were made prior to treatment and every 12 hours until negative, then once daily for 28 days. Blood films were considered negative if no parasites were seen in 200 oil immersion fields. Parasite counts per µl using Field's stained smears were determined by counting the number of asexual parasites per 200 white blood cells in thick blood films or per 1,000 red blood cells in thin blood films.

Clinical efficacy was assessed by determining the 28-day cure rate and by measuring parasite and fever clearance times (World Health Organization, 1973). The 28-day cure rate was defined as the proportion of patients who had no asexual parasitemia within seven days of initiation of treatment and who had no subsequent recrudescence within 28 days. Cure rates were evaluated on an intent-to-treat basis. The parasite clearance time (PCT) was the number of hours from the start of treatment until the first blood film became and remained negative for the next 24 hours. The fever clearance time (FCT) was the number of hours from the beginning of treatment until the patient's temperature decreased to 37.5ºC or lower, and remained below this temperature for at least the next 48 hours.

Standard descriptive and statistical analyses were conducted using version 6.04 of the EpiInfo software (Centers of Disease Control, Atlanta, GA). Comparisons were made using the chi-square and Student's *t*-test, and the Mann-Whitney *U* test, where appropriate.

RESULTS

A total of 120 patients (60 patients in each group) were enrolled in the study. One hundred and seventeen patients completed the study. Three patients (1 patient in group 1 and 2 patients in group 2, respectively) were lost to follow-up after day 7 due to social reasons, not to drug-related adverse effects. All patients were cured (no parasitemia and no fever) before they left the hospital. Only patients who were followed up for 28 days were included in calculations of drug efficacy.

Table 1 shows the baseline characteristics of the patients. There were 85 male and 35 female patients aged 15 to 63 years. There were no statistically significant differences between group 1 and group 2 patients with regards to any demographic, clinical or laboratory characteristics on admission.

Table 1
Baseline clinical and laboratory characteristics of the patients.

		Group 1 (n=60)	Group 2 (n=60)	p-value
Age (years)	Mean ± SD	25.6 ± 10.1	27.1 ±9.9	0.78
	(min-max)	(15-62)	(15-63)	
Sex (Male:Female)	No. (% Male)	42 : 18(70%)	43 : 17 (72%)	0.82
Duration of fever before admission (day)	Mean ± SD	5.3 ±6.1	6.0 ± 7.6	0.56
	(min-max)	(1-30)	(1-20)	
History of malarial infection	No. (%)	20 (33.3)	24 (40)	0.26
Hepatomegaly	No. (%)	5 (8.3)	7 (11.6)	0.52
Splenomegaly	No. (%)	5 (8.3)	6 (10)	0.91
Initial parasite count (per µl)	Geometric mean	6,754	9,883	0.22
	(min-max)	87-263,000	102-310,200	
Laboratory data				
Hematocrit (%)	Mean ± SD	34.8 ± 6.6	35.7 ±7.0	0.74
	(min-max)	(21-50)	(19-48)	
WBC count (x10^3/µl)	Mean ± SD	5.7 ± 2.4	5.2 ± 1.8	0.22
	(min-max)	(2.2-11.4)	(2.7-10.3)	
Total bilirubin (mg/dl)	Mean ± SD	1.4 ± 1.4	1.5 ± 1.3	0.72
	(min-max)	(0.36-8.3)	(0.30-9.56)	
Direct bilirubin (mg/dl)	Mean ± SD	0.5 ± 0.7	0.4 ± 0.6	0.61
	(min-max)	(0.10-4.10)	(0.09-3.80)	
SGOT (U/l)	Median	34.2	32.4	0.95
	(min-max)	(15-156)	(12-224)	
SGPT (Ul)	Median	38	40	0.46
	(min-max)	(12-218)	(15-492)	
Albumin (g/dl)	Mean ± SD	3.9 ± 0.5	4.0 ± 0.6	0.64
	(min-max)	(2.5-5.0)	(2.4-4.9)	
BUN (mg/dl)	Mean ± SD	14.9 ± 9.0	15.0 ±6.4	0.97
	(min-max)	(4.0-55.0)	(5.0-44.0)	
Cr (mg/dl)	Mean ± SD	0.9 ± 0.3	0.9 ± 0.2	0.81
	(min-max)	(0.56-2.12)	(0.65-1.50)	
G6PD deficiency	No. (%)	4 (6.1)	5 (10.2)	0.38

Group 1: was given artesunate 4 mg/kg/day for three days, (Gullin Pharmaceutical Corp, China 50 mg/tablet) together with mefloquine 8 mg/kg/day (250 mg/tablet) once a day for three days.
Group 2: was given artesunate 4 mg/kg/day for three days with mefloquine 15 mg/kg/day for the first day and 10 mg/kg/day for the second day simultaneously with artesunate.

Table 2
Parasitological and clinical outcomes.

		Group 1 (n=60)	Group 2 (n=60)
No. of patients with 28-day follow-up	No. (%)	59 (98.3)	58 (96.6)
No. of patients who dropped out	No. (%)	1 (1.6)	2 (3.3)
Fever clearance time (hours)	Median	32	33
	(min-max)	(4-162)	(4-173)
Parasite clearance time 100% (PCT 100%) (hours)	Mean ± SD	42.3 ± 10.7	43.3 ± 11.4
Parasite clearance time 90% (PCT 90%) (hours)	Mean ± SD	20.0 ± 7.1	18.9 ± 8.2
Parasite clearance time 50% (PCT 50%) (hours)	Mean ± SD	13.9 ± 6.4	12.1 ± 5.8
Cure rate at day 28 (%)	No. (%)	59 (100)	57 (98)
No. of recrudescence	No. (%)	0 (0)	1 (1.6)
Recrudescence	at day	0	26

On enrollment, patients in both treatment groups had common malaria symptoms, such as headache, asthenia, fatigue, fever, dizziness, nausea, vomiting, myalgia or anorexia. Most admission clinical manifestations were coincidental with high fever and gradually disappeared during the first 2-3 days of treatment. Some baseline laboratory parameters were affected by disease status but all returned to normal within 1-2 weeks.

All patients in this study responded promptly to both antimalarial regimens (Table 2). Very high cure rates were obtained with both regimens (100% for group 1 and 98% for group 2; p>0.05). One patient in group 2 recrudesced on day 26 (RI resistance pattern) and was successfully retreated with quinine-tetracycline for 7 days. No RII or RIII responses were seen in any study patient.

The mean PCT was rapid in both groups (42.3±10.7 hours *vs* 43.3±11.4 hours in group 1 and group 2, respectively (p=0.84)]. Parasites were cleared from the peripheral blood within 72 hours in all cases. Similarly, median times for fever clearance were rapid and not different between the two groups [32 hours *vs* 33 hours in group 1 and group 2, respectively (p=0.87)].

It was sometimes difficult to distinguish between the symptoms of acute malaria and drug-related side effects, but no serious adverse events were seen. A total of 22 patients experienced adverse effects (18.3%) but these were mild and most required no treatment. All patients suffering from adverse effects recovered without sequelae.

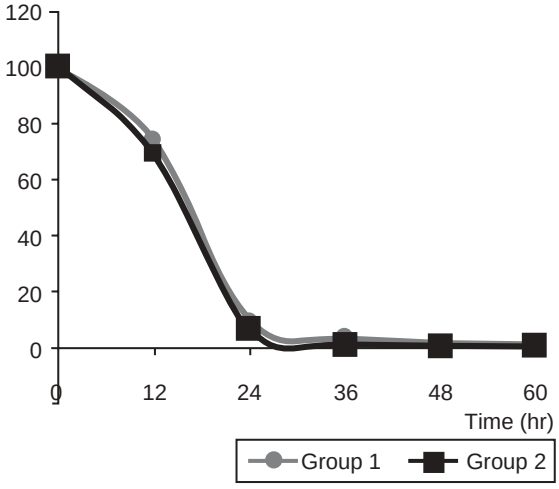

Fig 1–Mean parasite reduction times (%).

For all adverse effects the relationship to study medication was rated by the investigators as 'not related' or 'unlikely' to the investigated drugs. Possible side effects were mild headache, nausea, vomiting, diarrhea and dizziness. The proportions of patients in each group with these symptoms did not differ (4, 2, 3, 2, 4% *vs* 6, 3, 7, 1, 9% in group 1 and 2, respectively). Early vomiting (within 1 hour after dosing) occurred in two patients, both of whom were in group 2. No statistically significant changes in signs, symptoms or vital signs between the two groups were seen.

The overall tolerability of the treatment as assessed by the investigators on day 28 was judged to be 'very good' for almost all patients in both treatment groups. All pregnancy tests were negative.

DISCUSSION

With the deteriorating situation of multidrug resistant falciparum in Thailand (Looareesuwan *et al*, 1992a), attempts have been made to delay the development of resistance by the use of drug combinations. Combination treatment exploits the fact that different antimalarials have different mechanisms of action, and can act on distinct and separate biosynthetic pathways used by plasmodium parasites (Bloland *et al*, 2000; White *et al*, 1999). The use of two antimalarial agents can be highly effective. Of all the potential antimalarial drug combinations, the greatest safety and efficacy data are seen with artesunate and mefloquine (Looareesuwan *et al*, 1992c; McIntosh and Olliaro, 2001). Artemisinin combinations are highly effective at preventing resistance, because if only a single dose is given the total number of parasites surviving is still 0.01% of those present originally (White, 1999). After several treatment cycles, parasites may survive, but those few remaining parasites are then exposed to therapeutic blood mefloquine concentrations over a long period of time, since the half-life of mefloquine is 2 to 3 weeks. The high potency of artesunate that is potentially compromised by its short half-life is counterbalanced by the long half-life of mefloquine. The widespread introduction of the 3-day combined artesunate/mefloquine regimen for the treatment of falciparum malaria in Thailand in 1994 has led to almost 100% cure rates. No comparable period of sustained high efficacy for any antimalarial regimen has been reported in this population for the past 15 years (Nosten *et al*, 2000).

The overall safety profile of the combination of artesunate and mefloquine is good and reflects the well-known profiles of both drugs when used as monotherapy. The adverse effects pattern we saw matched those reported previously during other Asian studies. Our study showed that mefloquine can be safely administered from the first day of therapy onwards, thus greatly simplifying the treatment regimen. Increased mefloquine-related vomiting has been described in the literature with treatment begun on day 1 (Nosten *et al*, 1994; Price *et al*, 1998) but was not observed in our study of co-administration of both drugs. This combination of antimalarials was clearly tolerable. Only dizziness, but no major or specific central nervous system side effects were seen in either group. Symptoms disappeared, as did those associated with malaria. The side effect incidences were comparable between the two treatment groups and no specific neurological abnormalities were detected by neurological examinations.

Therefore, a 3-day treatment course with artesunate/mefloquine administered once daily is both highly effective and well tolerated in the treatment of acute, uncomplicated *P. falciparum* malaria in Thailand. This regimen produces rapid parasite and fever clearances, and its cure rate after 28 days of 100 % is similar to rates reported for combination treatment in previous Asian studies conducted during the last 10 years.

In conclusion, artesunate and mefloquine can be co-administered from the first day of therapy. This regimen is given once daily for 3 days and offers optimal efficacy while maintaining good safety and tolerability. This convenient dosing schedule is easy to prescribe and should enhance patient compliance and thereby contribute to limiting the development of drug resistance.

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