A CLINICAL TRIAL OF COMBINATION OF ARTESUNATE AND MEFLOQUINE IN THE TREATMENT OF ACUTE UNCOMPLICATED FALCIPARUM MALARIA: A SHORT AND PRACTICAL REGIMEN

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Abstract. The difficulties in treating drug-resistant falciparum malaria in Thailand are compounded by the necessity of giving antimalarials over long periods of time. The resultant fall in patient compliance not only lowers cure rates but also predisposes to the further spread of drug-resistance. Sequential treatment with artesunate given over 5 days followed by mefloquine produced 100% cure rates in previous study, but might not be a suitable regimen for field treatment. We conducted a clinical trial of a combination of artesunate and mefloquine given twice daily for 2 days in 150 patients with acute uncomplicated falciparum malaria. The dose of artesunate (200 mg) and mefloquine (312.5 mg) were given simultaneously in a separate package. All patients were admitted to a hospital in Bangkok for 28 days to exclude re-infection and monitor the possible adverse effects. One hundred and thirty patients completed the study with 28 days follow up. Twenty patients (13%) left the hospital prior to completion of follow-up for reasons unrelated to their treatment. Cure rate was 97% (126/130). There were no RII or RIII failures and all four patients with treatment failures were of the RI type. The mean parasite clearance time and fever clearance time were 46.4 and 42.5 hours, respectively. All patients were tolerated the combination drugs well and there were no serious toxic adverse reactions. The results indicate that combination of artesunate and mefloquine given twice daily for 2 days is effective and well tolerated in patients with acute, uncomplicated falciparum malaria and suitable as an alternative treatment for multidrug resistant falciparum malaria.

INTRODUCTION

Treatment of *Plasmodium falciparum* malaria in Southeast Asia is increasingly difficult and is a particular problem in Thailand. Resistance to all available standard antimalarials is well documented (Looareesuwan *et al,* 1992a). Quinine plus tetracycline for 7 days is a standard regimen for highly multidrug-resistant *P. falciparum* strains in Thailand. However, the cure rate with this combination varied between 90% and 98%, even though the drug administration was well supervised since the patients were treated in the hospital (Looareesuwan *et al,* 1992d; 1994). Both drugs have a short half-life (6-8 hours) necessitating frequent dosing. In addition, quinine causes predictable side effects (cinchonism). Both factors make treatment with quinine and tetracycline inconvenient and cause major problems with patient compliance, especially in the out-patient setting.

Mefloquine, a quinolinemethanol, has a long half-life (2-3 weeks) and can be given in a single dose. Treatment of falciparum malaria in Thailand with mefloquine (15 mg/kg) in adult patients produced a satisfactory response with cure rates of 98% in 1983 to 1986 (Harinasuta *et al,* 1987; Nosten *et al,* 1987). In 1990, the efficacy of mefloquine at the 15 mg/kg dose level had declined to 71% (Nosten *et al,* 1991). The higher dose of 25 mg/kg mefloquine produced cure rates of 98% in children and 74% in adults in a recent study (ter Kuile *et al,* 1992; Looareesuwan *et al,* 1994). At present, artesunate (600 mg total dose) followed by high-dose mefloquine (25 mg/kg) is used for the treatment of multidrug-resistant falciparum malaria on the eastern and western borders of Thailand.

Artesunate, an artemisinin derivative, has been licenced in Thailand for the treatment of falciparum malaria since 1990. It is a potent antimalarial drug that can reduce parasitemia by 90% within 24 hours after starting treatment. However, when given alone the recrudescence rate varies from 10 to

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100%, depending upon the dose and duration of treatment (Bunnag *et al,* 1991). Recent studies showed that a 5-day treatment course of artesunate (600 mg total dose) yielded a cure rate of 90% (Looareesuwan *et al,* 1992c). The cure rate was increased to 100% when mefloquine (25 mg/kg) was added after the end of artesunate (total dose 600 mg given over 5 days) treatment course (Looareesuwan *et al,* 1992b; c). However, a short course of artesunate (300 mg given in 2.5 days) followed by mefloquine (15 mg/kg) produced only 90% cure (Looareesuwan *et al,* 1993). Because pharmacokinetic data are lacking, regimens are empiric. Attempts were made to find a practical dosing regimen for artesunate and mefloquine in combination, with the aim of shortening the duration of treatment but maintaining efficacy. We report here a clinical trial of a simultaneous combination of artesunate and mefloquine given twice a day for 2 days for the treatment of acute uncomplicated falciparum malaria.

PATIENTS AND METHODS

Patients admitted to the Bangkok Hospital for Tropical Diseases, Thailand, between July 1997 and August 1998 were accepted into the study if they were diagnosed as having acute, uncomplicated falciparum malaria with parasite counts of 100 - 300,000 per µl of blood, were 15-60 years old, weighed 40-70 kg, gave informed consent to take part in the clinical investigation, and agreed to remain in hospital for 28 days. Reasons for exclusion were pregnancy, severe malaria (Warrell *et al,* 1990), or a history of antimalarial drug treatment within the preceding 2 weeks. This study was approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

All patients were admitted to hospital and remained there for 28 days (to exclude reinfection). Body temperature, pulse, and respiration rates were recorded every 4 hours. Signs and symptoms were evaluated every day for the first 7 days and weekly thereafter. Side effects were defined as signs and symptoms that first occurred or became more severe after treatment started. Cure was defined as the absence of a recrudescence during 28 days follow-up (World Health Organization, 1973).

Pretreatment investigations included full blood count, serum electrolytes, total and direct bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, albumin, globulin, and aspartate and alanine aminotransferases. These tests were repeated on days 7, 14, 21, and 28. Parasitological examination of thick blood films was done every 6 hours after treatment started until the blood films were negative; thereafter smears were performed daily. Blood films were considered negative if no parasites were seen in 200 oil immersion fields in a thick blood film. Parasite clearance time was the time from the start of treatment until the first time blood films were negative and remained negative for the next 24 hours. Fever clearance time was the time from the start of treatment until the oral temperature dropped to < 37.5°C and remained below this temperature during the next 48 hours.

Patients were treated with a combination of artesunate (Plasmotrim Lactab® 200 mg/tablet, MEPHA Ltd, Aesch-Basel, Switzerland) 200 mg (total dose 600 mg), together with mefloquine (Mephaquin Lactab® 250 mg/tablet MEPHA Ltd, Aesch-Basel, Switzerland) 312.5 mg (1 and $\frac{1}{4}$ tablets) given 12 hourly for 2 days (4 doses). Patients who vomited within one hour after drug administration were redosing.

RESULTS

One hundred and fifty patients were admitted to the study (98 males and 52 females). Clinical and laboratory characteristics are shown in Table 1. Sixty-four percent (96/150) were experiencing their first malaria attack. After treatment, twenty patients (13%) withdrew from the study (median 13.5 days, range 7 to 24 days) for social reasons unrelated to drug treatment or side effects. Before they left hospital they were well with negative parasitemias. A total of 130 patients remained in the hospital for a full 28 day-follow up. Only patients who were followed for 28 days were included in calculations of drug efficacy.

The cure rate was 97% (126/130) (Table 2). Recrudescences occurred between 26 and 28 days after treatment (median 27 days). Patients with recrudescent infections were treated with quinine plus tetracycline for 7 days. Parasite clearance time and fever clearance (Mean±SD) time were 46.4±13.0 hour and 41.5±30.5 hours respectively. After the start of treatment, 35 patients had at least one

Table 1

Clinical and laboratory characteristics of study groups before treatment.

No. of patients enrolled to study	150
Male/Female	98/52
Age (yr)	
Mean (SD)	26.6 (10.2)
Range	15-60
Mean (SD) height in cm	160.0 (8.1)
Mean (SD) weight in kg	54.0 (8.6)
Fever	
Duration before admission (days)	5.7 (5.4)
Highest fever before treatment (°C)	38.2 (0.9)
No. of patients with:	
Splenomegaly	40
Hepatomegaly	58
First malaria attack	96
Geometric mean parasite count (per μl)	9,030
Range	158-201,880
Laboratory data, mean (SD)	
Packed cell volume (%)	34.5 (8.2)
WBC count (per μl)	6,009 (2,275)
Blood urea (mg/dl)	15.1 (10.2)
Serum creatinine (mg/dl)	0.96 (0.45)
Total bilirubin (mg/dl)	1.6 (1.6)
Serum AST (IU/l)	42.9 (37.6)
Serum ALT (IU/l)	44.2 (58.7)
Albumin (g/dl)	4.0 (0.5)

Data given as mean(SD)
WBC = white blood count

parasite count greater than their initial parasite count. The median times to 50% and 90% parasite clearance were 15.1 and 22.8 hours respectively (Fig 1).

Signs or symptoms developing after treatment were headache in 13%, dizziness in 16%, nausea in 13%, abdominal pain in 9%, vomiting in 8% and diarrhea in 3%. These symptoms usually occurred within the first two days of treatment and coincided with high fever. It was difficult to distinguish between symptoms of acute malaria and drug-related side effects. Thirty-nine patients had increased transaminase(s) and/or total bilirubin levels prior to treatment. However, they all returned to normal within 1-2 weeks. Late increases (more than 3 times normal values) in transaminases, oc-

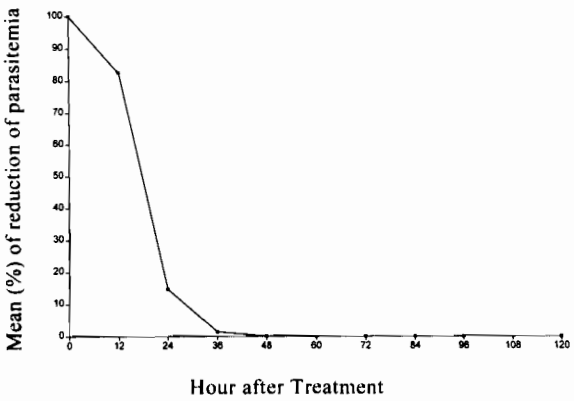

Fig 1–Mean of malaria parasite reduction (%) after treatment.

Table 2

Therapeutic responses.

No. of patients enrolled to study	150
No. of patients with 28-day follow up	130
No. of patients dropped out	20
RI response	4
Cure rate at 28 days (No. cured/total)	97% (126/130)
Recrudescence on day : Median	27
Range	26-28
Fever clearance time (h)*	
Mean (SD)	42.5 (30.5)
Range	0-152
100% parasite clearance time (h)**	
Mean (SD)	46.4 (13.0)
Range	18-93
90% parasite clearance time (h)	
Mean (SD)	22.8 (7.29)
Range	10-46
50% parasite clearance time (h)	
Mean (SD)	15.1 (7.2)
Range	6-35
Asexual forms of *P. vivax* positive during the 28-day follow up (%)	9/50 (6%)

* Fever clearance time (FCT) is the time between initiation of the antimalarial treatment and the time when the patient's temperature fell to 37°C or lower.

** Parasite clearance time (PCT) is the time between initiation of the antimalarial treatment and the time when the parasitemia reduce to 100%, 90% and 50% of the initial parasitemia for 100% PCT, 90% PCT and 50% PCT respectively.

curring two to three weeks after treatment, were found in 9 patients. The levels returned to normal before the patients were discharged. Other laboratory parameters were unremarkable.

DISCUSSION

Due to multi-drug resistance, the treatment of falciparum malaria in Thailand is a difficult problem (Looareesuwan *et al,* 1992a). Artemisinin and its derivatives are the most potent and rapidly acting antimalarial drugs. They reduce more parasite biomass in 48 hour than any other antimalarial drugs (White, 1997) and left the rest of parasites exposed to the second antimalarial drug. Artemisinin and its derivatives are remarkably well tolerated and, to date, no significant resistance has been reported either in clinical isolates or in laboratory experiments. Combinations of artesunate and mefloquine have proved highly effective even against multi-drug resistant *P. falciparum* (Price *et al,* 1997; van Vugt *et al,* 1998). On the north-western border of Thailand, where the most resistant *P. falciparum* exist, the use of combination chemotherapy has retarded the progression of mefloquine resistance. There are many reasons for this. First, combinations give high cure rates because three day's of treatment with an artemisinin derivative eliminates most of the infection, and the residual of parasites are exposed to the more slowly eliminated mefloquine (White, 1997). These residual parasites are exposed to mefloquine alone. Second, with the rapid reduction in the parasite load, the selective pressure for the emergence of mutants with reduced mefloquine sensitivity is lower (White, 1998). Third, artesunate reduces gametocyte carriage (Price *et al,* 1996; Looareesuwan *et al,* 1998). Recrudescent (*ie* resistant) infections are

associated with increased gametocyte carriage rates which provides a selection pressure to the spread of resistance (Price *et al,* 1996; Handunnetti *et al,* 1996; Looareesuwan *et al,* 1998). This is prevented by artesunate. These benefits are particularly important in areas of unstable transmission such as Thailand. Therefore, combination therapy should slow the evolution of drug resistance. In addition, they benefits of the combinations is the rapid therapeutic response and patients are able to return to school or work earlier. However, at present, there is no commercially available finished product using the two combined antimalarials drugs (artesunate and mefloquine) as finished marketing in age (FMI). We therefore used the two individual drugs given simultaneously to patients in this study.

Although sequential treatment of artesunate followed by mefloquine have been reported in previous studies with effective result (Looareesuwan *et al* 1992b,c; 1994; 1996), the compliance may reduce due to long period of drug administration. New short course drug regimens that provide curative outcome is therefore needed for practical use.

In this study with a short course, practical regimen, with treatment with artesunate and mefloquine given simultaneously twice a day for 2 days gave an effective result with 97% cure rate and improved clinical response with rapid parasite and fever clearance times.

The regimen may be considered an alternative treatment for acute uncomplicated falciparum malaria in areas of multidrug resistance and be particularly useful for treating patients in rural areas where the period of hospital stay should be as short as possible.

ACKNOWLEDGEMENTS

We are grateful to nurses and staff members of the Hospital for Tropical Diseases for their help and support. The study was supported by Mepha Ltd, Aesch-Basel, Switzerland and the budget of the Thai Government.

REFERENCES

Bunnag D, Viravan C, Looareesuwan S, Karbwang J, Harinasuta T. Clinical trial of artesunate and artemether in multidrug resistant falciparum malaria in Thailand: a preliminary report. *Southeast Asian J Trop Med Public Health* 1991; 22: 380-5.

Handunnetti SM, Gunewardena DM, Pathirana PPSL, Ekanayake K, Weerasinghe S, Mendis KN. Features of recrudescent chloroquine-resistant *Plasmodium falciparum* infections confer a survival advantage on parasites and have implications for disease control. *Trans R Soc Trop Med Hyg* 1996; 90: 563-7.

Harinasuta T, Bunnag D, Vanijanonta S, *et al.* Mefloquine, sulfadoxine, and pyrimethamine in the treatment of symptomatic falciparum malaria : a double-blind trial for determining the most effective dose. *Bull WHO* 1987; 63: 363-7.

Looareesuwan S, Harinasuta T, Chongsuphajaisiddhi T. Drug resistant malaria with special reference to Thailand. *Southeast Asian J Trop Med Public Health* 1992a; 23 : 621-34.

Looareesuwan S, Kyle DE, Viravan C, *et al.* Treatment of patients with recrudescent falciparum malaria with a sequential combination of artesunate and mefloquine. *Am J Trop Med Hyg* 1992b; 47: 794-9.

Looareesuwan S, Vanijanonta S, Viravan C, Wilairatana P, Charoenlarp P, Andrial M. Randomized trial of mefloquine alone and artesunate followed by mefloquine for the treatment of acute uncomplicated falciparum malaria. *Ann Trop Med Parasitol* 1994; 88: 131-6.

Looareesuwan S, Vanijanonta S, Viravan C, *et al.* Randomised trial of mefloquine-tetracycline, and quinine-tetracycline for acute uncomplicated falciparum malaria. *Acta Tropica* 1994; 57:47-53.

Looareesuwan S, Viravan C, Vanijanonta S, *et al.* Treatment of acute uncomplicated falciparum malaria with a short course of artesunate followed by mefloquine. *Southeast Asian J Trop Med Public Health* 1993; 24: 230-4.

Looareesuwan S, Viravan S, Vanijanonta S, *et al.* A randomised trial of mefloquine, artesunate and artesunate followed by mefloquine in acute uncomplicated falciparum malaria. *Lancet* 1992c; 1: 821-4.

Looareesuwan S, Viravan C, Vanijanonta S, Wilairatana P, Pitisuttithum P, Andrial M. Comparative clinical trial of artesunate followed by mefloquine in the treatment of acute uncomplicated falciparum malaria: two and three-day regimens. *Am J Trop Med Hyg* 1996; 54: 210-3.

Looareesuwan S, Wilairatana P, Vanijanonta S, Kyle D, Webster K. Efficacy of quinine-tetracycline for acute uncomplicated falciparum malaria in Thailand. *Lancet* 1992d; 1: 369.

Looareesuwan S, Wilairatana P, Gemperli B, Gathman I Royce C. Phase III trial in Thailand comparing a new oral combination antimalarial CGP 5669 with oral mefloquine in the treatment of *Plasmodium*

falciparum malaria. *Am J Trop Med Hyg* 1998; (in press).

Nosten F, Imvithaya S, Vincenti M, *et al.* Malaria on the Thai-Burmese border : treatment of 5192 patients with mefloquine-sulfadoxine-pyrimethamine. *Bull WHO* 1987; 65: 891-6.

Nosten F, ter Kuile F, Chongsuphajaisiddhi T, *et al.* Mefloquine-resistant falciparum malaria on the Thai-Burmese border. *Lancet* 1991; 1: 1140-3.

Price RN, Nosten F, Luxemburger C, *et al.* Effects of artemisinin derivatives on malaria transmissibility. *Lancet* 1996; 347: 1654-8.

Price RN, Nosten F, Luxemburger C, *et al.* Artesunate/ mefloquine treatment of multi-drug resistant falciparum malaria. *Trans Soc Trop Med Hyg* 1997; 91: 574-7.

ter Kuile F, Nosten F, Thieren M, *et al.* High-dose mefloquine in the treatment of multidrug-resistant falciparum malaria. *J Infect Dis* 1992; 166: 1393-40.

van Vugt M, Brockman A, Gemperli B, *et al.* Randomized comparison of artemether-benflumetol and artesunate-mefloquine in treatment of multi-drug-resistant falciparum malaria. *Antimicrob Agents Chemother* 1998; 42: 135-9.

Warrell DA, Molyneux ME, Beales PF. Severe and complicated malaria. 2nd ed. *Trans R Soc Trop Med Hyg* 1990; 84 (suppl 2): 1-65.

White NJ. Assessment of the pharmacodynamic properties of antimalarial drugs *in vivo*. *Antimicrob Agents Chemother* 1997; 41: 1413-22.

White NJ. Preventing antimalarial drug resistance through combinations. *Drug Resist Updates* 1998; 1: 3-9.

World Health Organization. Advances in malaria chemotherapy. *WHO Tech Rep Ser* 1973; 529: 30-5.