AN OPEN RANDOMIZED CLINICAL TRIAL OF ARTEKIN® *VS* ARTESUNATE-MEFLOQUINE IN THE TREATMENT OF ACUTE UNCOMPLICATED FALCIPARUM MALARIA

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Abstract. Malaria remains a major cause of morbidity and mortality in tropical countries and subtropical regions in the world. Southeast Asia has the most resistant malaria parasites in the world, which has limited treatment options in this region. In response to this situation, short-course artemisinin-based combination therapies (ACTs) have been developed. The combination of dihydroartemisinin (DHA) and piperaquine (PQP) in the form of Artekin® has been developed as an alternative to established combinations, such as artesunate-mefloquine, primarily to reduce treatment costs and toxicity. We conducted a study comparing a standard treatment for acute uncomplicated falciparum malaria (artesunate 4 mg/kg/day together with mefloquine 8 mg/kg/day oral route once a day for 3 days) (Group A) and a combination of dihydroartemisinin 40 mg and piperaquine 320 mg in the form of Artekin® given once a day for 3 days (Group B) to determine safety, efficacy, and tolerability. One hundred and eighty patients were randomly enrolled at the ratio of 1:2 into groups A:B. All patients had rapid initial clinical and parasitological responses. There were no significant differences in fever clearance time or parasite clearance time between both groups. The 28-day cure rates were high, at 100% and 99%, in groups A and B, respectively. We conclude that Artekin® was as effective and well-tolerated as artesunate-mefloquine, and can be used alternatively as the current treatment for multidrug-resistant *P. falciparum* malaria.

INTRODUCTION

Malaria is the major cause of mortality and morbidity in the tropical and subtropical regions of the world. An estimated 300-500 million persons suffer from malaria every year and more than 1million die annually. The majority of these cases and deaths, particularly those among children, occur in Sub-Saharan Africa. Unlike some of the other acute diseases, such as encephalitis, meningitis, and most of the chronic diseases, patients with severe malaria can recover completely without any long-term effects if treated promptly and correctly. Therefore, rationalization and standardization of treatment of cases of severe or uncomplicated malaria at different levels of health care is important. Deaths can be reduced by the effective use of standard treatment. Patients who require hospitalization, and those who need intensive care, can be identified promptly and treated before they die or develop complications. The adoption of this standard-management approach can reduce the mortality and morbidity of malaria (WHO, 2004).

Approximately 800-1,000 malaria cases are admitted to the Bangkok Hospital for Tropical Diseases annually. These include *P. falciparum* (51%), *P. vivax* (46%), mixed infections of *P. falciparum* and *P. vivax* (2%), a few cases of *P. malariae* and occasional cases of *P. ovale*. Admitted patients are all treated with antimalarial regimens and most of them are enrolled for clinical trial (Faculty of Tropical Medicine, 2004).

Resistance to antimalarial drugs is increasing nearly everywhere in the tropical world, confounding global attempts to “Roll Rack Malaria”

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(Nosten and Brasseur, 2002). Southeast Asia has the most resistant malaria parasites in the world, which has limited treatment options in this region (WHO, 2001). In Thailand, the treatment of acute uncomplicated falciparum malaria is becoming more difficult because of increasing resistance to all of the antimalarial drugs, except the artemisinin derivatives (Wilairatana *et al*, 2002). To combat the further spread of resistance, it is generally accepted that combinations of antimalarial drugs that include an artemisinin derivative should be used, and, if possible, that preparations should be formulated in a single tablet (Hien *et al*, 2004).

The artemisinin derivatives (artesunate and the recently developed dihydroartemisinin, which is short-acting but powerful drug) have been studied extensively in the treatment of falciparum malaria in Thailand, and are well-tolerated. Their main drawback is that conventional courses (3-5 days) are associated with high rates of recrudescence, typically >25%. In addition, there is the risk that parasite resistance will develop when antimalarial drugs are used alone (Warhurst, 1999). Because artemisinin derivatives are now the first-line treatment for multidrug-resistant falciparum malaria in many tropical countries, the appearance of artemisinin-resistant *Plasmodium falciparum* would have serious implications. Thus development of suitable combinations of an artemisinin compound with a second drug is therefore a priority (WHO, 2001). At present, artesunate has been registered by the Thai FDA for use in the treatment of falciparum malaria.

Mefloquine is another antimalarial drug, which is better tolerated than quinine and can be administered during a day, but resistance to mefloquine has developed when used alone. Furthermore, in Thailand where multidrug resistance is encountered, a high dose (25 mg/kg) of mefloquine is recommended for use as a combination with other short-acting antimalarial drugs (Nosten *et al*, 1991). Recently, clinical trials have shown that artesunate combined with mefloquine is effective and well-tolerated (Looareesuwan *et al*, 1992, 1994, 1996; Price *et al*, 1997); therefore, this regimen has been chosen for treating multidrug resistant falciparum malaria in Thailand. However, some patients cannot tolerate the adverse effects of mefloquine.

Piperaquine phosphate (1,3-bis[1-(7-chloro-4'-quinolyl)-4'-piperazinyl]) phosphate) replaced chloroquine as the recommended treatment for *Plasmodium falciparum* malaria in China in 1978 and was used extensively for mass prophylaxis and treatment. Reported adverse events are generally similar to those observed with chloroquine, although pruritus is uncommon. (Tropical Medicine Institute, 2003). Piperaquine was proved to be effective and well-tolerated, and no cross-resistance with chloroquine was observed (Chen *et al*, 1982). More recently, piperaquine has been used as part of short-course artemisinin-based combination oral therapies designed to have high cure rates and few side effects, and to reduce malaria transmission (Denis *et al*, 2002; Davis *et al*, 2005).

Artekin® (compound dihydroartemisinin), a combination of dihydroartemisinin 40mg, piperaquine 320mg per tablet (Batch No. 20011204 Mfg. 120401 Exp. 120403), supplied free of charge by Holleykin Pharmaceutical Co Ltd, Guangzhou, China is claimed to be highly effective. In addition, this combination is well-tolerated and convenient to use (3 days' treatment). This compound has been on clinical trial and proved safe and well-tolerated in China, Vietnam, Lao PDR, Cambodia, and elsewhere (Karunajeewa *et al*, 2003; Hien *et al*, 2004). We propose here a clinical trial of Artekin® *vs* artesunate and mefloquine (a standard regimen for treatment of multidrug-resistant falciparum malaria in Thailand) at the Bangkok Hospital for Tropical Diseases, to determine efficacy, safety, and tolerability.

MATERIALS AND METHODS

Study site and recruitment procedures

All patients were included who fulfilled the inclusion criteria (acute uncomplicated falciparum malaria, either male or female; if female, pregnancy test negative before enrolment into the study, positive asexual forms of *P. falciparum* in blood smear, weight > 40 kg and age > 14 years, ability to take oral medication, agreement to stay in hospital for at least 28

days). Informed consent for the study was obtained from the patients or their guardians before enrolment into the study. The patients were admitted to the Bangkok Hospital for Tropical Diseases for 28 days to exclude reinfection and to assess the safety and efficacy of Artekin® and artesunate plus mefloquine. We excluded severe malaria according to WHO criteria (WHO, 2000), severe vomiting not allowing oral medication, pregnancy or lactating female, significant concomitant systemic diseases (for example systemic bacterial infections, liver and/or kidney insufficiencies, chronic disease or severe malnutrition), diseases requiring therapy other than malaria, ingestion of other antimalarials in the past 14 days or presence of urine sulfonamides or 4-aminoquinolones. Clinical evaluation, including neurological examination focused on brain stem, cerebellar function, muscle strength in all limbs, extraocular and facial muscle strength, deep tendon reflexes, and finger-to-nose tests; parasite counts were performed 12-hourly until negative, then daily for 28 days. Malaria parasite counts per microliter were obtained by calculation against white blood cell counts for a thick film. Geometric mean parasites were used as the standard method. Blood films were considered negative if no parasites were seen in 200 oil-immersion microscopic fields. Fever clearance time was taken as the period from the start of treatment until the oral temperature decreased to 37.5ºC and remained below this temperature for the next 48 hours. Side-effects were defined as signs and symptoms that occurred or became more severe after treatment started. Cure rate at day 28 (cured patients/evaluable patients x 100%) was defined as the absence of parasite recrudescence during 28 days of follow-up. For any RI, RII, or RIII failure (WHO, 1973), standard antimalarial drugs of the hospital would be given. Adverse events would also be treated by standard procedures at the Bangkok Hospital for Tropical Diseases. This study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Study drug administration

An open randomized clinical trial of Artekin® *vs* Artesunate-Mefloquine was conducted at the Bangkok Hospital for Tropical Diseases, Mahidol University. Upon admission to the ward, patients were randomly treated at a ratio of 1:2 in groups A:B, as follows:

Group A: AM: Artesunate (4 mg/kg/day) was given by oral route once a day for 3 days together with mefloquine 8mg/kg/day for 3days.

Group B: Artekin: Artekin® (2 mg/kg/day of dihydroartemisinin and 15 mg/kg/day piperaquine) was given by oral route once a day for 3 days (Artekin® 1 tab contains DHA 40 mg + piperaquine 320 mg).

All patients were treated symptomatically as indicated (*eg* intravenous fluid and antipyretics.) according to the standard practice in the hospital. In cases of RI, RII, or RIII responses (WHO, 1973), other antimalarial drugs (*eg* quinine plus tetracycline for falciparum malaria, and chloroquine followed by primaquine for vivax malaria) were used as indicated. Patients who vomited within one hour after drug administration were re-dosed.

Monitoring for safety

Patients were given physical examinations and adverse reactions during the study were recorded with the date and time at which they occurred and disappeared. Adverse effects were assessed on the basis of non-suggestive questioning by the study investigators; these included gastrointestinal, central nervous system, cardiovascular and dermatological effects, as well as other changes possibly attributable to the study drugs. Routine blood investigations (hematology and biochemistry) and urinalysis were performed prior to (Day 0) and weekly for 4 weeks of the study period.

Statistical analysis

Statistical analysis was performed using the Analyze It Add-Ins for Excel for Windows. All the p-values reported were from 2-tailed testing, and the statistically significant level was set at 0.05. Data distribution normality was assessed using the Schapiro-Wilks test. Data were expressed as mean and SD. Two statistical tests were performed–chi-square to test differences between 2 groups of qualitative variables, and independent *t*-test to test the difference between 2 groups of quantitative variables, on demographics and baseline laboratory data (Tabachnick *et*

al, 2001).

RESULTS

A total of 180 patients were enrolled into this trial. All pregnancy tests in the female patients were negative. Around 90% of patients completed the study as planned. Demographic clinical data and pretreatment laboratory characteristics are shown in Table 1. One hundred and thrity-five male and 45 female patients aged 14-65 years participated in this trial. There were no significant differences in the distributions of demographic, clinical or laboratory data between the two treatment groups.

At enrolment, the patients in both treatment groups showed common malaria symptoms, such as headache, asthenia, fatigue, fever, nausea, vomiting, myalgia, and anorexia. Most clinical manifestations present on admission gradually disappeared during the first few days of treatment and coincided with high fever. Some baseline laboratory parameters were affected by disease status. However, they all returned to normal within 1-2 weeks.

Nineteen patients (6 and 13 in each group) did not complete the 28-day follow-up due to social reasons not related to adverse effects. Thus, 161 of 180 patients (89.5%) completed the 28-day study. No patient in either group deteriorated through clinical or biochemical

Table 1
Clinical and laboratory characteristics of study groups before treatment.

	Group A (n = 60)	Group B (n = 120)
Male/Female	49/11	86/34
Age (yr)		
Mean (SD)	26.5 (10.6)	24.3 (8.5)
Range	14-65	14-58
Mean (SD) height in cm	161.5 (9.3)	160.5 (8.1)
Mean (SD) weight in kg	55.6 (10.9)	51.8 (9.7)
Fever [Mean(SD)]		
Duration before admission (days)	5.6 (5.3)	5.3 (4.4)
Highest fever before treatment (C)	38.2 (0.9)	38.3 (1.0)
No. of patients with:		
Splenomegaly	3	5
Hepatomegaly	12	19
Urine positive for drugs[a]	0	0
First malaria attack	29	32
Geometric mean parasites		
Count (per µl)	4,645	3,759
Range	13 - 102,500	17 - 190,860
Laboratory data [mean (SD)]		
Packed cell volume (%)	35.8 (5.1)	36.0 (6.0)
WBC count (per µl)	6,239 (4,593)	5,579 (1,776)
Blood urea (mmol/l)	14.2 (7.0)	15.3 (7.2)
Serum creatinine (µmol/l)	0.8 (0.2)	0.9 (0.2)
Total bilirubin (µmol/l)	1.4 (0.9)	1.5 (1.1)
Serum AST	38.1 (22.6)	42.7 (52.7)
Serum AAT	39.8 (34.4)	38.6 (31.8)
Albumin (mg/l)	3.7 (0.5)	3.6 (0.5)
Alk PO_4	134.9 (56.4)	140.5 (100.4)

WBC = white blood count; AST, AAT= aspartate and alamine aminotransferases (U/l)
Alk PO4 = alkaline phosphatase (U/l); [a]Sulfonamides and 4-aminoquinolones

Table 2
Therapeutic responses.

	Group A (n = 60)	Group B (n = 120)
No. (%) of patients who dropped out	6 (10%)	13 (10.8%)
No. of patients with 28-day follow-up	54	107
No. (%) cured at 28 days	54 (100%)	106 (99%)
Recrudescence on days	-	21
Fever clearance time (hours)		
Mean (SD)	25.2 (28.4)	24.8 (24.7)
Range	4-100	4-124
Parasite clearance time (hours)		
Mean (SD)	39.6 (13.7)	35.0 (16.2)
Range	11-84	4-74

changes after treatment. Parasitological and clinical responses are shown in Table 2. All patients in this study showed a prompt response to both antimalarial regimens (Fig 1). The cure rates at 28 days' follow-up of group A and B were 100% and 99%, respectively. There were no significant differences in fever-clearance or parasite-clearance times between the two treatment groups. No patients had RII or RIII failures. Only one patient in the Artekin® treatment group had recrudescence on day 21 of the study period. This patient was administered rescue antimalarial chemotherapy according to the hospital's standard regimen. Therefore, all patients were parasitologically negative on discharge from our hospital.

Mean times for parasite clearances in each treatment group were fast; however, there were no statistical significance between the two treatment groups [39.6 ± 13.7 hours and 35.0 ± 16.2 hours in groups 1 and 2, respectively, (p=0.72)]. The parasites were all cleared from peripheral blood smears within 84 hours. There was no statistically significant difference between the fever clearance times of the two treatment groups (p=0.67).

No fatal case occurred. No patient had vomiting related to the drugs. There were no major adverse effects and no neurologic or neuropsychiatric manifestations during treatment or during the 28-day follow-up period. Some minor symptoms, such as nausea, headache, and dizziness occurred in group A (4, 3, 2 patients) and group B (5, 4, 4 patients), respectively. However, these signs and symptoms could not be differentiated from malaria symptoms as they disappeared 1-4 days after treatment and while fever subsided. In addition, there was no serious adverse event reported during the study.

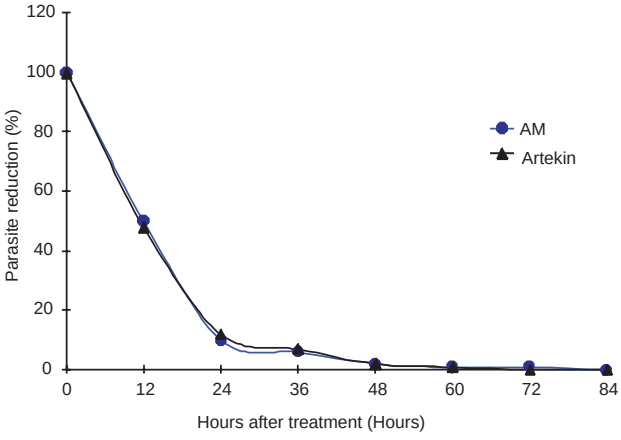

Fig 1–Percentage of malaria parasite reduction after treatment.

DISCUSSION

In Thailand, *Plasmodium falciparum* is resistant to chloroquine and there has been a decline in sensitivity to mefloquine (Brockman *et al*, 2000). The use of artemisinin derivatives has been central to successful malaria control efforts in Thailand, Vietnam, and Cambodia (Looareesuwan *et*

al, 1997; Denis *et al*, 2002; Hien *et al*, 2004). Artemisinin derivatives are potent, rapidly acting antimalarials that can reduce parasitemia by more than 90% within 24 hours in uncomplicated malaria cases. However, the rate of recrudescence within 28 days when used alone can be as high as 10-25% depending upon dosage, duration of treatment, and severity of disease (Hien *et al*, 1991; Li *et al*, 1994). These drugs are often combined with other long-acting antimalarials, such as mefloquine, (in this study, DHA was combined with piperaquine, to improve efficacy and compliance). The rationale for using the combination is the same as for the standard multidrug treatment for tuberculosis, patients with HIV, and most cancers. The rapid parasite-killing ability of the artemisinin derivatives accelerates the therapeutic response, prevents dangerous early treatment failure in cases of high-grade resistance, reduces parasite biomass, and reduces gametocyte transmission (Looareesuwan *et al*, 1999). The benefit of adding an appropriate and suitable long-acting drug is the prevention of recrudescence by killing residual parasites, reducing the risk of resistant mutant surviving parasites. The long-acting antimalarial might protect the artemisinin derivative in low transmission areas. Combined administration of artemisinin derivatives and mefloquine in different dosages and durations has been studied in uncomplicated malaria in many countries. This combination is now a standard treatment for multidrug-resistant falciparum malaria in Thailand (Wilairatana *et al*, 2002). However, some disadvantages of using artesunate-mefloquine have been observed (*eg* some patients could not tolerate mefloquine).

The combination of DHA and piperaquine in the form of Artekin® was developed as an alternative to established combinations, such as artesunate-mefloquine, primarily to reduce treatment costs and toxicity. Our hospital-based study has shown that a combination of dihydroartemisinin and piperaquine is effective and well-tolerated by Thai adults with acute uncomplicated *Plasmodium falciparum* malaria. Like the previous studies (Denis *et al*, 2002; Hien *et al*, 2004), most of the patients treated with Artekin® in this study improved clinically and had negative parasitemia on the blood smear by the third day of treatment. All patients responded satisfactorily to both treatment regimens. The present study showed a high total cure rate (99-100%) in both groups. Artekin® showed similar cure rates to the standard treatment (artesunate-mefloquine). However, it remains unclear whether the improved cure rate is due to the synergistic effect of dihydroartemisinin and piperaquine. No patient died during this study. Compared with the 3-day combination artesunate-mefloquine treatment, Artekin® is given only 3 doses in 3 days. The shorter period for Artekin® is now on clinical trial and might be better, with a high chance of complete treatment course and improved compliance. This combination may serve as an alternative regimen in treating uncomplicated falciparum malaria. Artekin® has more advantages in these respects and more importantly, since the drug was produced as a fixed combination, shorter duration of treatment and potentially lower cost than artesunate-mefloquine. Other long-acting drugs, other than piperaquine combined with artemisinin derivatives are under development.

In conclusion, the results of this study indicate that Artekin® is effective and well-tolerated. Artekin® may be an alternative treatment to the standard combination of artesunate-mefloquine for multidrug-resistant uncomplicated falciparum malaria in Thailand. However, more studies with special groups (children, pregnant women) and field trials, and pharmacokinetic studies to guide rational dosing regimens, are needed to elicit more information on Artekin®.

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REFERENCES

Brockman A, Price RN, van Vugt M, *et al*. *Plasmodium falciparum* antimalarial drug susceptibility on the northwestern border of Thailand during five years of extensive artesunate-mefloquine use. *Trans R*

Soc Trop Med Hyg 2000; 94: 537-44.

Chen L, Qu FY, Zhou YC. Field observations of the antimalarial piperaquine. *Chin Med J* 1982; 95: 281-6.

Davis TM, Hung TY, Sim IK, *et al*. Piperaquine: a resurgent antimalarial drug. *Drugs* 2005; 65: 75-87.

Denis MB, Devis TM, Hewitt S, *et al*. Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian children and adults with uncomplicated falciparum malaria. *Clin Infect Dis* 2002; 35: 1469-76.

Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Annual report, 2004: 80.

Hien TT, Dolecek C, Mai PP, *et al*. Dihydroartemisinin-piperaquine against multidrug-resistant *Plasmodium falciparum* malaria in Vietnam: randomized clinical trial. *Lancet* 2004; 363: 18-22.

Hien TT, Tam DT, Cuc NT, Arnold K. Comparative effectiveness of artemisinin suppositories and oral quinine in children with acute falciparum malaria. *Trans R Soc Trop Med Hyg* 1991; 85: 201-11.

Karunajeewa H, Lim C, Hung TY, *et al*. Safety evaluation of fixed combination piperaquine plus dihydroartemisinin (Artekin,) in Cambodian children and adults with malaria. *Br J Clin Pharmacol* 2003; 57: 93-99.

Li GQ, Guo XB, Fu LC, *et al*. Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. *Trans R Soc Trop Med Hyg* 1994; 88: S5-6.

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* 1992; 47: 794-9.

Looareesuwan S, Vanijanonta S, Viravan C, *et al*. Randomised 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, Viravan C, Vanijanonta S, *et al*. 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, Chokejindachai W, *et al*. A randomized, double-blind, comparative trial of a new oral combination of artemether and benflumetol (CGP 56697) with mefloquine in the treatment of acute *Plasmodium falciparum* malaria in Thailand. *Am J Trop Med Hyg* 1999; 60: 238-43.

Looareesuwan S, Wilairatana P, Vanijanonta S, *et al*. Monotherapy with artesunate for uncomplicated falciparum malaria: a comparison of 5-day and 7-day regimens. *Acta Trop* 1997; 67: 197-205.

Nosten F, Brasseur P. Combination therapy for malaria. *Drug* 2002; 62: 1315-29.

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

Price RN, Nosten F, Luxemburger C, *et al*. Artesunate-mefloquine treatment of 1967 patients with multidrug resistant falciparum malaria. *Trans R Soc Trop Med Hyg* 1997; 91: 574-7.

Tabachnick BG, Fidell LS. Using multivariate statistics. 4th ed. USA: Allyn & Bacon, 2001: 41-5.

Tropical Medicine Institute, Guangzhou University of Traditional Chinese Medicine, Chinese. Documentation of Artekin for new drug registration. 2003.

Warhurst DC. Drug resistance in *Plasmodium falciparum* malaria. *Infection* 1999; 27: S55-8.

Wilairatana P, Krudsood S, Chalermrut K, *et al*. An open randomized clinical trial of Artecom *vs* Artesunate-Mefloquine in the treatment of acute uncomplicated falciparum malaria in Thailand. *Southeast Asian J Trop Med Public Health* 2002; 33: 519-24.

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

World Health Organization. Antimalarial drug combination therapy: report of a WHO Technical Consultation. Geneva: WHO, April 2001: 35.

World Health Organization. Guidelines for treatment of severe malaria in hospitals. 2004. [Accessed 2005 May]. Availableat: at: URL: http://mosquito.WHO.int/docs/sm_SEARO. pdf

World Health Organization. Management of severe malaria: a practical handbook. 2nd ed. Geneva: WHO, 2000.