codon 76, with a change from lysine to threonine, has been invariably found in CQ-resistant laboratory strains and also in CQ-resistant field isolates from Southeast Asia, including Lao PDR, Thailand; and South America (Fidock *et al*, 2000; Wongsrichanalai *et al*, 2002). As several investigators have reported that the haplotype of *pfcrt* position 72-76 was CVIET in Thailand (Chen *et al*, 2001; Labbe *et al*, 2001), our results also indicated that all isolates tested in the present study area had a CVIET haplotype. On the other hand, point mutations in *pfmdr1*, especially at codon 86, have been known to associate with decreased CQ susceptibility (Duraisingh *et al*, 1997). In this study, 14 of 18 (78%) isolates that were successfully examined for CQ resistance, had a Y86 mutation (4 of these 18 isolates were mixed haplotype). Thirty-seven of 39 (95%) isolates that analyzed PCR-RFLP had Y86 mutation. These results suggested that there is a correlation between CQ resistance, and *pfcrt* T76 and *pfmdr1* Y86 mutations.

Mefloquine-resistant falciparum malaria has increased and presents a real threat to the control of malaria on the Thai-Myanmar border (Boudreau *et al*, 1982; Harinasuta *et al*, 1983). In this study, 8 of 16 (50%) isolates had MF-resistance and they also had the *pfmdr1* Y86 mutation. However, PCR-RFLP demonstrated that MF-susceptible isolates also had Y86 mutation; thus, the correlation between MF-resistance and *pfmdr1* mutations was not seen. Recently, several studies have reported that increased copy numbers of *pfmdr1* correlated with MF resistance (Pickard *et al*, 2003; Price *et al*, 1999, 2004). To understand more of the relationship between MF-resistance and *pfmdr1*, it may be necessary to assess the *pfmdr1* copy numbers.

In conclusion, highly CQ-resistant falciparum malaria parasites that have *pfcrt* CVIET haplotype were prevalent in Thai-Myanmar border areas. In addition, correlations between CQ resistance and mutations of *pfcrt* (T76) and *pfmdr1* (Y86) were observed. It is necessary to assess the new molecular techniques in the surveillance of antimalarial drug resistance in various epidemiological settings because the associations among *pfcrt* haplotype, *pfmdr1* copy numbers, and the levels of drug-resistance are still unclear. Further studies are also needed to clarify whether the drug susceptibility of *P. falciparum* might be influenced by the treatment measures against other human malaria parasites that are not falciparum malaria.

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RESEARCH NOTE

PYRIMETHAMINE-SULFADOXINE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN LAO PDR

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Abstract. A 28-day *in vivo* treatment trial to evaluate the efficacy of pyrimethamine/sulfadoxine (Fansidar©, PS) was conducted in 21 Lao patients with uncomplicated *Plasmodium falciparum* malaria. Sixteen patients (76%) were completely cured with PS without any reappearance of asexual stage parasitemia during the follow-up examination. On the other hand, 5 patients (24%) failed to respond to this trial medication, resulting in recrudescence of asexual stage *P. falciparum* malaria. PS resistance resulted in higher prevalence of post-treatment gametocytemia, 25% gametocyte carriers among PS sensitive cases versus 75% of the resistant cases. These findings suggest that although the level of PS resistance is still valid for treatment of malaria in the study area of Lao PDR, post-treatment induction of gametocytemia among resistant cases may result an increase in transmission rate of PS resistant falciparum malaria.

INTRODUCTION

Lao PDR is a developing country in Southeast Asia bounded by borders with Myanmar, Cambodia, China, Thailand, and Vietnam. Malaria is the most serious public health problem in Lao PDR, accounting for an estimated 1,561 deaths from 1998 to 2002, as recorded by Center of Malariology, Parasitology and Entomology (CMPE), Vientiane. Although the limited use of impregnated bed nets and other preventive measures, such as health education, have been playing a big role to gradually reduce the morbidity and mortality due to malaria in recent years, there has been growing concern due to increasing frequency of treatment failure over the period of past decade. Because an alarmingly increasing emergence of resistant falciparum malaria to chloroquine, the recommended first-line antimalarial agent for uncomplicated malaria in the country, has become evident in a series of *in vivo* and *in vitro* studies in Lao PDR (Tawil, 1977; Giboda *et al*, 1992; Pillai *et al*, 2001; Mayxay *et al*, 2003), has been suggested that a revised national policy for treatment of malaria is needed. Indeed, local doctors are now facing enormous troubles in giving treatment to malaria patients with chloroquine, as treatment failure due to resistance not only complicates the disease but also increases the transmission rate of resistant malaria.

Under these circumstances, a combination of pyrimethamine and sulfadoxine (PS), the recommended second-line antimalarial agent in Lao PDR, has become the drug of choice for treat-

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ment of malarial patients in the country. Although there are very few reports to support or refute the current use of PS, local and sporadic observations suggest that PS-resistant falciparum malaria is also developing. In view of the deteriorating multidrug-resistant malaria in the countries bordering Lao PDR (Gomes *et al*, 1978; White, 1992; Smithuis *et al*, 1997), the importation of PS-resistant malaria from the neighboring countries is a possibility, especially because of recent population movements across the porous borders.

With this view in mind, the present study was undertaken to assess the efficacy and appropriateness of PS so as to justify whether it has potential for prevention and treatment of malaria in a selected region of Lao PDR. The study also focused on the influence of PS treatment on gametocytemia. Although this study is confined to *in vivo* treatment trial only, it should deserve attention as an essential step for further studies on the efficacy of PS, including genotype analysis to understand the mechanism of resistance.

MATERIALS AND METHODS

With approval from Ministry of Health, Lao PDR, the present study was conducted from February to August 2003 in close co-operation with senior CMPE officials, who helped in the selection of study sites as well as in field settings, language interpretation and technical expertise. Based on a preliminary small-scale survey, this study was conducted during the rainy season in the field settings of rural areas of Lao Ngam and Khongsedon districts of Saravan Province, a malaria endemic area with moderate to low transmission, located about 800 km south of Vientiane. The residents of the study areas belong to two ethnic groups, namely, Lao Luom (lowland) and Lao Theung (upland), who live by cultivation and farming.

RESULTS

A total of 1,192 people were examined for active case detection of *Plasmodium falciparum* by microscopy using thick and thin smears of finger prick blood. Twenty-nine samples were positive for *P. falciparum*, one of which was also identified as mixed-infection with *P. vivax*. Eight cases were positive for *P. vivax* by microscopic examination of Giemsa's stained blood film. Overall prevalence encompassing *P. falciparum* (2.4%), *P. vivax* (0.7%) and a mixed infection (0.1%) was 3.1%.

Among 29 *P. falciparum* positive cases, 8 cases were excluded from *in vivo* treatment trial according to the exclusion criteria, including pregnancy and positive cases diagnosed by the presence of gametocytes only. The remaining twenty-one cases (10 males and 11 females with age of 2-45 years, 2 adults and 19 children) met the inclusion criteria for the *in vivo* treatment study with signs and symptoms of acute uncomplicated *P. falciparum* malaria (fever less than 39.5°C on enrolment or a history of fever within the previous 24 hours), single infection with *P. falciparum*, and initial parasitemia (asexual stage parasites) of more than 1,000 per microliter blood. The cases were, with informed consent, administered PS (1.25 mg pyrimethamine/kg body weight) under strict supervision (day 0) and were followed up on days 2, 3, 7, 14, 21 and 28 for axillary temperature measurements, thick and thin blood smears and blood spots on filter paper. Giemsa's stained blood smears were read by microscopists of CMPE and filter paper blood was used for analysis of MSP-1 and MSP-2 genes of *P. falciparum* to distinguish recrudescence from re-infection. The efficacy of treatment was determined by blood examination in which no asexual parasitemia was detected after the treatment.

Table 1 summarizes the results of the 28-day *in vivo* treatment trial with PS. Sixteen (76%) of 21 *P. falciparum* patients recruited in the *in vivo* trial were completely cured with PS without any reappearance of asexual parasitemia during the follow-up examination. Five (24%) patients failed PS treatment, suggesting the possibility of infection due to recrudescence. For 3 of the 5 resistant cases, the parasitologic failure to respond to PS was graded as R III resistance, as manifested by the recurrent parasitemia within the first week of the follow-up examination. The other resistant cases had recurrence during days 14-28, which was consistent

Table 1
Results of a 28-day *in vivo* therapeutic trial with pyrimethamine/sulfadoxine against *Plasmodium falciparum* malaria in Saravan Province of Lao PDR.

Features	Result
Number of patients	21
Mean age (Range)	11 (2-45) years
Percent male	47.6
Parasitologic response	
Sensitive	16/21 (76%)
Resistance (total)	5/21 (24%)
RI/RII resistance	2/21 (10%)
RIII resistance	3/21 (14%)

Treatment failure was defined by the presence of asexual stage parasites in the blood during the follow-up period. RI/RII resistance indicates late treatment failure while RIII indicates early treatment failure.

with RI/ RII resistance, as identified by the analysis of MSP-1 and MSP-2 genes of *P. falciparum* using isolates from filter paper samples (data not shown).

DISCUSSION

Because of the emergence of high degree of chloroquine-resistant falciparum malaria in Lao PDR, PS seems to be a good candidate for replacement therapy. Data on the efficacy of PS, however, are not available to support this view. Only recently Schwöbel *et al* (2003) demonstrated that PS resulted in 17.9% treatment failure in a 14-day *in vivo* trial in Attapu Province, Lao PDR. This finding is not so inconsistent with our 28-day trial with PS which resulted in 24% treatment failure. Even if we had conducted our study for 14 day, it would have produced 14% (3 out of 21) treatment failure. Although we cannot extrapolate these findings to other areas of Lao PDR, knowledge from unpublished sources (CMPE record) indicates that PS resistance, even with its developing stage, exists within a safe range.

On the other hand, the question is that PS resistance is associated with post-treatment induction of gametocytemia. In the present study, we observed 80% (4 out of 5) gametocyte carriers among patients having therapeutic failure to PS, as compared with that of only 25% (4 out of 16) gametocyte carriers among patients having an adequate therapeutic response. In an epidemiological study in Gambia, von Seidlein *et al* (2001) also observed the association of PS treatment with high prevalence of post-treatment gametocytemia. However, the study failed to make clear whether resistance is the prime (essential) contributor to post-treatment gametocytemia. Further studies are necessary to establish the association of PS resistance with post-treatment induction of gametocytemia, since gametocytes do not contribute to disease pathology but it has impact on the spread of malaria. Induction of gametocytemia by treatment failure may worsen the situation by spreading resistant falciparum malaria. The high gametocyte prevalence among PS resistant patients suggests that the continuous use of PS is in contrast to conventional treatment policy. Because of the poor socio-economic infrastructure of Lao PDR, it is quite difficult for the policy makers to go beyond PS (or chloroquine), which is easily available and affordable. von Seidlein *et al* (2001) have shown that PS combined with artesunate at a single dose cleared both sexual and asexual parasitemia in Gambian patients, and this can be chosen as an affordable cost-effective antimalarial, provided that the therapy is effective in Lao PDR.

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1: Southeast Asian J Trop Med Public Health. 2005 Sep;36 (5):1085-91. Related Articles, Links

An open randomized clinical trial of Artekin vs artesunate-mefloquine in the treatment of acute uncomplicated falciparum malaria.

Tangpukdee N, Krudsood S, Thanachartwet W, Chalermrut K, Pengruksa C, Srivilairit S, Silachamroon U, Wilairatana P, Phongtananant S, Kano S, Looareesuwan S.

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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.

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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 death 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. At the present, the combination of Dihydroartemisinin (DHA) and Piperaquine (PQP) in the form of Artekin® was 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-Mefloquine) (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 1:2 into group A:B. All patients had rapid initial clinical and parasitological responses. There were no significant differences in fever clearance time and parasite clearance time between both groups. The 28 day cure rates were high as 100% and 99% in the both groups respectively. We conclude that Artekin® was effective and well-tolerated as artesunate-mefloquine, the current treatment in this area of multidrug-resistant P. falciparum malaria.

INTRODUCTION

Malaria is the major cause of mortality and morbidity in the tropical and subtropical regions in the world. An estimated 300-500 million persons suffer from malaria every year and more than 1million die each year. Majority of these cases and deaths particularly those in children occur in Sub Saharan Africa. Unlike some of the other acute diseases such as encephalitis, meningitis, and most of the chronic diseases, patients of 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. It has several advantages deaths can be reduced by effective use of standard treatment procedures. 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 approach of standard management can reduce the mortality and morbidity from malaria (WHO, 2004).

Approximately 800 – 1,000 malaria cases are admitted to Bangkok Hospital for Tropical Diseases annually. These include *P. falciparum* (51%), *P. vivax* (46%), mixed infections of *P. falciparum* and *P. vivax* (2%), 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" (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, treatment of acute uncomplicated falciparum malaria is becoming more difficult because of increasing resistance to all 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 a recently developed, dihydroartemisinin which is short acting but powerful drug) has been studied extensively in the treatment of falciparum malaria in Thailand, are well tolerated. Their main drawback is that conventional

courses (3-5 days) are associated with high rate 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. The 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 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 of artesunate combination with mefloquine has proved effective and well tolerated (Looareesuwan *et al*, 1992; Looareesuwan *et al*, 1994; Looareesuwan *et al*, 1996;Price *et al*, 1997), therefore this regimen has been chosen for treatment of multidrug resistant falciparum malaria in Thailand. However, some patients can not tolerate 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 pruitus is uncommon. (Tropical Medicine Institute, 2003). Piperaquine 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 a high cure rate, to have 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 for high effective. In addition, this combination is well tolerated and convenient for use (3 days treatment). This compound has been on clinical trials and proved safe and well tolerated in

China, Vietnam, Laos, Cambodia and else where (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 the safety, tolerability, and efficacy.

MATERIALS AND METHODS

Study site and recruitment procedures

All patients who fulfilled inclusion criteria (acute uncomplicated falciparum malaria, either male or female; if female, pregnancy test has to be negative before enrolment to the study, positive asexual forms of *P. falciparum* in blood smear, weight more than 40 kg and age more than 14 years, ability to take oral medication, agreement to stay in the hospital for at least 28 days). Informed consent to the study was obtained from patients, or their guardians, before enrolment to 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 except malaria, ingestion of other antimalarials in the past 14 days or presence of urine sulphonamides or 4-aminoquinolones. Clinical evaluation including neurological examination focused on brain stem, cerebellar function, muscle strength in all limb, extraocular and facial muscle strength, deep tendon reflexes, and finger-to-nose tests, and also parasite count were performed 12 hourly until negative then daily for 28 days. Malaria parasite count per microliter was obtained by calculation against the white blood cell count for a thick film. Geometric mean parasites were used as a 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 37.5 °C 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. If there is RI, RII, or RIII failure (World Health Organization, 1973), standard antimalarial drugs of the hospital will be

given. Adverse events will also be treated as 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 ratio of 1:2 into groups A:B as follows:

Group A: **AM:** Artesunate (4mg/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 40mg + Piperaquin 320mg)

All patients were treated symptomatically as indicated (e.g. 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 (e.g. 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 redosed.

Monitoring for safety

Patients were physically examined 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 include gastrointestinal, central nervous, cardiovascular, dermatological effects, as well as other changes possibly attributable to the study drugs. Routine blood investigations (hematology and biochemistry), and urinalysis were performed prior (Day 0) and weekly for 4 weeks of the study period.

Statistical analysis

Statistical analysis was performed by using the Analyze It Add Ins Excel for Windows. All the *P-values* reported are from 2-tailed test and the statistically significance level was set at 0.05. The distribution of data was assessed for normality using the Schapiro-Wilks test. Data were expressed, as means and SD. Two statistical tests were performed. We used chi-square analyses to test differences between 2 groups of the qualitative variables and independent t-test to test the difference between 2 groups of the quantitative variables on demographics and baseline laboratory data. (Tabachnick *et al,* 2001)

RESULTS

A total of one hundred and eighty patients were enrolled in this trial. All pregnancy tests in female patients were negative. Around 90% of patients completed the study as planed. Demographic clinical data and pretreatment laboratory characteristics are shown in Table 1.There were 135 male and 45 female patients aged 14 to 65 years old participated in this trial. There were no significant differences in the distribution of demographic, clinical and laboratory data between the two treatment groups.

At enrolment, patient 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 (6 and 13 patients in each group) patients did not complete the 28-days follow up due to social reasons not related to adverse effects. Thus, 161 patients out of 180 patients (89.5%) completed the 28-day study. No patients were deteriorated in clinical or biochemical changes after treatment in both groups. Parasitologic and clinical responses are shown in Table II. All patients in this study showed a prompted response to both antimalarial regimens (Figure 1). The cure rates at 28 days of follow-up were 100% and 99% respectively for the both treatment groups. There were no significant differences in fever clearance time and parasite clearance time between both treatment groups. No patients had RII or RIII failures. Only one patient in Artekin® treatment group had recrudescence on day 21 of study

period. The patient who drug failed to clear parasitemia was given the rescue antimalarial chemotherapy according to the hospital's standard regimen. Therefore, all patients had parasitologically negative at the time discharged from our hospital.

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

No death occurred. No patients had vomiting related to the drugs. There were no major adverse effects and no neurologic or neuropsychiatric manifestations during treatment and 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 in group B (5, 4, 4 patients) respectively. However, these signs and symptoms could not be differentiated from malaria symptoms as they were disappeared between 1-4 days after treatment and while fever subsided. In addition, there was no serious adverse event reported during the study.

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 the artemisinin derivatives has been central to successful malaria control efforts in Thailand, Vietnam and Cambodian (Denis *et al*, 2002; Hien *et al*, 2004; Looareesuwan *et al*, 1997). Artemisinin derivatives are potent, rapidly acting antimalarials that can reduce parasitemias 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 combined with piperaquine, to improve efficacy and compliance). The rationale using of the combination is as standard treatment of multidrug treatment for taberculosis, patients with HIV and most cases of cancers. The rapid killings of parasitemias of artemisinin derivatives is accelerate the therapeutic response, prevent dangerous early treatment failures in case of high grade resistance,

reduce the parasite biomass and reduce gametocyte transmission (Looareesuwan et *al*, 1999). The benefit of adding appropriate and suitable long action drug is prevent recrudescence by killing residual parasites, reduce the chance of a resistant mutant surviving and in addition the long acting antimalarial might protect the artemisinin derivative in low transmission areas. Combined administration of artemisinin derivatives and mefloquine in different dosages and duration had 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 might be seen (e.g. 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 an effective and well tolerated by Thai adults with acute uncomplicated *plasmodium falciparum* malaria. Like a previous study (Denis et al, 2002; Hien et al, 2004) most of the patients whose treated by Artekin® in this study improved clinically and were parasite negative on the blood smear by the third day of treatment. The present study, all patients responded satisfactorily to the both treatment regimens. As well as, the present study shows a high total cure rate (99-100%) in the both groups. Artekin® showed similar cure rate to the standard treatment (artesunate-mefloquine). However at present, it has remained unclear whether the improve cure rate due to synergistic effect of the synergy dihydroartemisinin and piperaquine. There was no fatal patient in the study. Comparing with 3-day combination of artesunate-mefloquine treatment, Artekin® is given only in 3 days with 3 doses. The shorter period of Artekin® is now on clinical trial and might be better and have high chance of complete treatment course and improved compliance. This combination may serve as alternative regimens for treatment of uncomplicated falciparum malaria. The Artekin® has more advantage on these issues and more importantly since the drug was produced as a fix combination, less duration of treatment and possibly lower cost than artesunate-mefloquine. Other long acting drugs combined with artemisinin derivatives are under invention.

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 in treatment of multidrug resistant uncomplicated falciparum malaria

such as in Thailand. However, additional and more studies in special groups (in children, pregnant women, and field trials) and pharmacokinetic studies to guide rational dosing regimens are needed in order to get more informations of Artekin® in general practice.

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

	Group A	Group B

	(n = 60)	(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*	0	0
First malaria attack	29	32
Geometric mean parasites		
count (per μl)	4,645	3,759
Range high	102,500	190,860
Low	13	17
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 (umol/L)	0.8 (0.2)	0.9 (0.2)
Total bilirubin (umol/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 PO_4 = alkaline phosphatase (U/L)
*Sulphonamides and 4-aminoquinolones