

THERAPEUTIC EFFICACY OF ARTESUNATE IN *PLASMODIUM VIVAX* MALARIA IN THAILAND

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Abstract. Our previous study showed that *in vitro* susceptibility of *Plasmodium vivax* to chloroquine has significantly decreased in Thailand within the past two decades. Thus, the evaluation of alternative antimalarials for treatment of vivax malaria is needed. The aim of this study was to examine parasitological and clinical efficacy of an artemisinin derivative (artesunate) for the treatment of vivax malaria in patients who were admitted to the Bangkok Hospital for Tropical Diseases. We randomly allocated patients aged 12-56 years to receive 3.3mg/kg (adult dose 200 mg) on the first day, and for the next four days each patient was given 1.65 mg/kg orally (adult dose 100 mg), total dose = 600 mg. After the five-day course of artesunate, primaquine was given: a single oral dose of 15mg for 14 days. A total number of 42 patients received treatment. All participants were followed up for 28 days. In all the cases, both parasitemia and fever were resolved rapidly; the mean fever clearance time and parasite clearance time, 14.6 and 36.7 hours, respectively, showed that therapeutic response to artesunate was better than that of chloroquine. The 14-day cure rate was 100%, but re-appearance of parasitemia was seen in two patients on days 21 and 25 following treatment, respectively. These two cases of failure rate should be considered as true relapse rather than recrudescence, since the relapse interval in Southeast Asian vivax malaria according to recent findings seems to be 3 weeks after start of treatment, if primaquine is not given or an inadequate amount is given. In conclusion, artesunate might be useful in treatment of vivax malaria, causing a good blood schizontocidal effect. However, to prevent emerging resistance it should never be used alone.

INTRODUCTION

Malaria is considered to be one of the world's worst health problems; it is responsible for 300 million cases of clinical disease, and jeopardizes the health of 2.4 billion people in more than 90 countries - some 40% of world's population. Malaria kills an estimated 1.5-2.7 million people every year (Schwartlander, 1997). Malaria remains one of the major infectious diseases in Thailand, especially in the areas that border Cambodia and Myanmar, where multidrug-resistant *Plasmodium falciparum* is found. Throughout the past decade the number of malaria cases has var-

ied between 168,370 (1992) and 83,767 per year (1996); almost 47% of the cases during this period were caused by *Plasmodium vivax*; the annual parasite incidence (API) was 3.1-1.17 per 1,000 population. Overall, a declining trend in the number of malaria cases has been seen in recent years (Fig 1). *Anopheles dirus* and *An. minimus* are the most important vectors (Malaria Division, 2001). Indoor insecticide spraying, the distribution of impregnated bednets, limited biological control, diagnosis and treatment, and health education in schools and general communities are the main malaria control measures applied in the endemic areas (Malaria Division, 2001).

While chloroquine-resistant *P. falciparum* was described almost 40 years ago in Thailand (Harinasuta *et al*, 1965), the second most common cause of malaria, *P. vivax*, has until recently remained sensitive to this valuable anti-malarial

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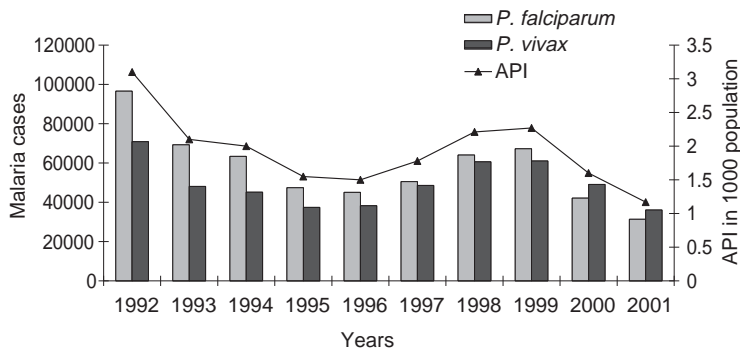


Fig 1—Trend of annual malaria cases and API in Thailand during 1992-2001.

agent. Chloroquine (CQ), a 4-aminoquinoline antimalarial, has been the drug of choice for more than 40 years in many parts of the world (Collins and Jeffery, 1996). CQ is well absorbed, well tolerated and inexpensive; moreover, it rapidly and effectively eradicates blood-stage parasites, usually within 36-72 hours. However, in the past decade CQ-resistant *P. vivax* has been reported from several countries, including Indonesia, Brazil, Myanmar, India, and Colombia (Whitby *et al*, 1989; Baird *et al*, 1991; Canessa *et al*, 1992; Marlar *et al*, 1995; Singh, 2000; Soto *et al*, 2001). In Colombians infected with *P. vivax*, 11% failed to respond to treatment with the standard regimen of chloroquine (Soto *et al*, 2001).

Artesunate (ART) is one of the artemisinin (qinghaosu) derivatives, a promising new class of antimalarials, derived from *Artemisia annua*, a medicinal plant that has been used for the treatment of fever in China for more than 1,500 years (Klayman, 1985). Moreover, neither adverse effects nor significant changes in laboratory test results from this drug have been observed (Nguyen *et al*, 1993; Alecrim *et al*, 2000). Chloroquine-resistant vivax malaria is emerging and the evaluation of alternative antimalarials for the treatment of vivax malaria is required now; time is short (Marsh, 1998). In vivax malaria, parasites are not sequestered in the microcirculation and, therefore, any reduction in parasitemia after treatment is a direct reflection of antimalarial activity.

The present clinical trial had the primary objectives of evaluating the parasite clearance time (PCT), fever clearance time (FCT), and prob-

able reappearance of parasitemia within 28 days; the subjects were hospitalized patients who had vivax malaria, and they were treated with artesunate.

MATERIALS AND METHODS

Study site

The study was conducted between September 2001 and May 2002 in the Department of Clinical Tropical Medicine and the Hospital for Tropical Diseases Faculty of Tropical, Bangkok. The study

was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

Patients

Forty-two patients were enrolled in the study; the mean age (\pm SD) was 24 years (\pm 8.8). There were 29 (69%) male and 13 (31%) female subjects; the body weight range was 40-75 kg. Prior to admission, written informed consent was obtained from all the adult participants and from the parents of the child participants.

Inclusion and exclusion criteria

Patients were enrolled if they met the following inclusion criteria: no history of malaria during the past 24 months; no use of antimalarials in the past 2 months. The exclusion criteria were: mixed infection with *P. vivax* and *P. falciparum*; pregnancy; unwillingness to be hospitalized for at least 28 days.

Treatment with artesunate

All patients were treated with artesunate (50 mg base tablets; Guilin Pharmaceutical Works, Guangxi, China). On the first day each patient was given 3.3 mg/kg orally (adult dose 200 mg); on each of the next four days, each patient was given 1.65 mg/kg orally (adult dose 100 mg). The artesunate was given as single doses with food. After the five-day course of artesunate, primaquine (7.5 mg tablets; Thai Government Pharmaceutical Organization) was given: a single oral dose of 15 mg base for 14 days. All medication was administered under supervision.

In vivo assessment and parasite count

The *in vivo* efficacy of artesunate was evalu-

ated using the World Health Organization standard extended test (Bruce-Chwatt, 1981). Patients were monitored in hospital for 28 days in order to note relevant clinical features, eliminate the possibility of reinfection, and diagnose reappearance of parasitemia. Peripheral blood smears were made at the time zero and then every 12 hours until free of malaria parasites, after which they were made daily until day 28. The following three variables were chosen to demonstrate parasite clearance: the time from the start of antimalarial treatment until the asexual malaria parasite count had fallen by 50% (PC_{50}) and then by 90% (PC_{90}) of the admission value; the PCT was defined as the time from the start of treatment until blood smears were negative for asexual parasites for at least 48 hours. Body temperature was recorded every 4 hours; FCT was defined as the time from the start of treatment until the oral temperature dropped below 37.5°C and remained below this temperature for the next 48 hours. Variables to define the rate of parasite reduction were the ratio of the parasite count before treatment to the counts at 24 hours (PRR_{24}) or at 36 hours (PRR_{36}). Parasitemia (asexual parasites/ μ l blood) was calculated from the number of asexual parasites per 200 white blood cells (thick films) or per 10,000 red blood cells (thin films). Total white cell and red cell counts were also performed. Blood films were considered to be negative if no parasites were seen in 200 high power fields of a thick blood smear.

Statistical analysis

For comparison of the means *t*-test was used, association between fever clearance, parasite clearance, and rates of parasite reduction were measured by using Spearman's rank correlation coefficient. All statistical analysis were performed with the statistical computing package SPSS (version 8.0 for Windows).

RESULTS

The mean parasite density of the blood samples was 11,953 asexual parasites per microliter. The mean FCT for all 42 vivax malaria patients treated with artesunate was 14.6 hours. Only 3 patients showed a FCT of over 32 hours. The mean PCT was 36.7 hours (range 22-67). The

Table 1
PRRs for patients with *Plasmodium vivax* malaria treated with artesunate.

	No. of patients	Mean	Range	SD
PRR_{24}	37	2,202	35-18,090	4,572
PRR_{36}	16	9,605	87-35,420	11,497

mean parasite clearance times for 50 and 90% of the parasites (PC_{50} and PC_{90}) were 10.8 hours (range 4-21 hours) and 14.1 hours (range 6-24 hours), respectively. Following treatment, the calculated PRR_{24} and PRR_{36} for all patients varied widely, ranging from 35 to >35,000, Table 1. Since, in almost all the patients, parasites had been eradicated within 48 hours of treatment, PRR_{48} could not be calculated. Reappearance of parasitemia was seen in 2 patients on days 21 and 25 respectively. Administration of same regimen of ART and 22.5 mg primaquine (PQ) for 14 days as antirelaps, to each of these two resulted in clearance of parasitemia with no further *P. vivax* paraitemias in the following 28 days. Comparing the patients who had a reappearance of parasitemia with those who did not, there was no significant difference ($p > 0.05$) in the admission parasitemia (means of 7,534 vs 15,536/ μ l), PCT [means of 37 hours; (SD=1.41) vs 36.9 hours; (SD=9.18)], and FCT (means of 13 hours vs 14.85 hours). There was no significant correlation or association in PCT and FCT (Spearman's rho = 0.189), but there was a significant correlation between PCT and PRR_{24} (Spearman's rho = -0.454; $p < 0.05$).

DISCUSSION

In recent years, drug resistance has become an important issue in malaria control (Fevere *et al*, 1999). Multi-drug resistance, which is widespread in Thailand, has been attributed mainly to selection pressure on *P. falciparum*, the wide availability of drugs, poor compliance, and patients' self-treatment in subtherapeutic doses (Harinasuta *et al*, 1965).

Throughout the second half of the last century, and until recently, CQ has been the drug of

choice for the clinical treatment of vivax malaria. Whereas the resistance of *P. falciparum* to CQ developed rapidly, the resistance of *P. vivax* to CQ is emerging slowly - some three decades after the resistance of *P. falciparum* - in many parts of the world (Nomura *et al*, 2001). Thailand too is at risk from CQ-resistant *P. vivax*. Mutations in a digestive vacuole protein encoded by 13-exogen, *Pfprt*, were shown recently to have a central role in *P. falciparum* CQ resistance. However, the mechanism of CQ-resistance in *P. vivax* is not completely understood; the molecular basis of *P. vivax* CQ-resistance is likely to differ from that of *P. falciparum* (Nomura *et al*, 2001).

Although there has been no reported decrease in the clinical efficacy of CQ in Thailand, the trend of declining *P. vivax* sensitivity (*in vitro*) during the last two decades (Hamedi *et al*, 2003) raises a question: is it time to revise the treatment of vivax malaria? The data from the present study suggest that artemisinin derivatives (especially artesunate) could be used instead of CQ, but in order to prevent an unacceptable rate of recrudescence and the development of resistance in the future, artemisinin derivatives should be administered in combination with another blood schizonticide that has an independent mode of action. White *et al* (1999) have argued that the treatment of malaria with a single drug should no longer be regarded as ethical. Combination chemotherapy can stop the progressive trend of declining susceptibility of *P. vivax* to CQ. However, although monotherapy with artemisinin derivatives is safe, it is probable that adverse effects may result from combinations of artemisinin with other antimalarials. Accordingly, research into the safety of combination antimalarial chemotherapy is needed. Recently, the treatment of vivax malaria patients with a conventional regimen of CQ and primaquine in the Bangkok Hospital for Tropical Diseases produced a PCT of 64 hours and a FCT of 30 hours (Pukrittayakamee *et al*, 2000). However, as the present study shows, ART gives more rapid parasite clearance and significantly a quicker resolution of fever ($p < 0.001$); in other words, the therapeutic effect of ART is better than that of CQ in the treatment of *P. vivax*. Nevertheless, assessment of the therapeutic response to vivax malaria is complicated by the

appearance of relapses. These results from dormant liver stages (hypnozoites) of the parasite, which are insensitive to all antimalarials except 8-aminoquinolines. For this reason, radical treatment of vivax malaria requires treatment with PQ. The relapse interval in Southeast Asian vivax malaria has traditionally been reported to be 6 weeks (Collins and Jeffery, 1996). However, it is evident that when vivax malaria is treated with effective short half-life antimalarial drugs and PQ is not given, then the infection reappears on average 3 weeks after the start of treatment (Baird *et al*, 1997). PQ is the only available chemotherapeutic agent for eradication of hypnozoite form of *P. vivax*. Conventional dose of PQ for eradication of hypnozoite is 15 mg daily for 14 days. But the Chesson strain of *P. vivax* is well recognized in Southeast Asia and is known to require higher doses of PQ (Warrell, 1993). Bunnag *et al* (1994) estimated that 17.5% of *P. vivax* infections acquired in Thailand are resistant to conventional doses of PQ. Subsequently it was confirmed by Doherty *et al* (1997). Since, ART is a short half-life antimalarial, in spite of administration of PQ, 2 cases of relapses occurred, both were retreated with the same dosage regimen of ART but a higher dose of PQ. No further parasitemia was observed in the following 28 days.

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