THE EFFICACY OF COMBINED MEFLOQUINE-ARTESUNATE VERSUS MEFLOQUINE-PRIMAQUINE ON SUBSEQUENT DEVELOPMENT OF PLASMODIUM FALCIPARUM GAMETOCYTEMIA

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Abstract. An open randomized controlled study of mefloquine-artesunate and mefloquine-primaquine for the treatment of uncomplicated Plasmodium falciparum malaria was carried out in Kanchanaburi in the Saiyok District in western Thailand. Weekly parasite counts from thick and thin blood films were done for six weeks. The gametocyte carriage rate was calculated and compared between the two treatment groups. Gametocytes on presentation, recrudescence, and reinfection were the significant factors associated with subsequent development of gametocytemia.

It is the increased propensity of recrudescence to produce gametocytes that drives drug resistance. The results of this study confirmed that the complete eradication of asexual forms of P. falciparum by effective antimalarial treatment, but not by combination treatment with primaquine, is the most effective means to prevent subsequent gametocytemia.

INTRODUCTION

The progression of antimalarial drug resistance is the major problem confronting the National Malaria Control Program in Thailand. On the western and eastern borders of this country, Plasmodium falciparum has developed resistance to chloroquine, sulfadoxine-pyrimethamine, and mefloquine. The increase in mefloquine resistance is expected to spread to the nearby malaria-endemic areas. Long-term studies on the Thai-Burmese border showed that the incidence of P. falciparum malaria was reduced dramatically after the general Thai-Burmese border showed that the incidence of the nearby malaria-endemic areas. Long-term studies on the Thai-Burmese border showed that the incidence of P. falciparum malaria was reduced dramatically after the general introduction of a combination of an artemisinin derivative and mefloquine for the treatment of uncomplicated P. falciparum malaria in that area. Results of the studies from this area suggested that the transmission of P. falciparum malaria decreased because artemisinin derivatives reduced gametocytemia in these patients.

Drug therapies that inhibit gametocytogenesis or kill mature gametocytes have the potential to interrupt transmission of P. falciparum malaria. Infection of the anopheline mosquito vector occurs when human peripheral blood gametocyte densities are above or close to the limit of microscopic detection. Therefore, transmissibility can be assessed from a peripheral blood smear. Primaquine, an 8-aminoquinoline, is an antimalarial drug effective against mature gametocyte of P. falciparum. We report the results of a study comparing the efficacies of artesunate and primaquine when combined with mefloquine on gametocyte carriage, a measure of the transmission potential of P. falciparum.

MATERIALS AND METHODS

This study was an open, multicenter, randomized, controlled trial at the Saiyok Hospital and other six health centers in the Saiyok District in Kanchanaburi in western Thailand. The Saiyok District has a population of approximately 35,000 living in an area of malarious hill forest close to the Thai-Burmese border. Transmission of malaria is low and seasonal. Nearly all P. falciparum malaria infections are symptomatic. Health officers participating in the study were able to prepare, stain, and interpret the thick blood films. They were trained and supervised regularly by the Malarial Center of the Ministry of Public Health of Thailand. Parasite counts were made on Giemsa-stained thick and thin blood films.

All admission and follow-up thick and thin blood films of patients included in this study were recounted by the experienced microscopist at the Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine at Siriraj Hospital, Mahidol University, Bangkok, Thailand. If the count on the thick blood film exceeded 1,000 parasites per 500 white blood cells, the thin blood film result (expressed as the number of parasitized erythrocyte per 100 red blood cells) was recorded. The number of gametocytes was counted only on thick blood films. All parasite counts were done by technicians unaware of the patients’ drug treatment. The study was reviewed and approved by the Ethical Review Subcommittee of the Ministry of Public Health, Thailand.

Patients with symptomatic, microscopy-confirmed P. falciparum malaria were enrolled into the study after written informed consent were obtained from adult patients and from the parents or legal guardians of minors. Exclusion criteria included patients with a history of previous antimalarial treatment within one week, pregnant and lactating women, patients who were unlikely to come back for follow-up, and patients with severe or complicated P. falciparum malaria as determined by the 2000 criteria of the World Health Organization. After entering into the study, patients were randomly allocated to receive either mefloquine-artesunate (MA) (mefloquine: 25 mg base/kg in two divided doses [Me pha, Aesch-Basel, Switzerland] plus artesunate: 4 mg/kg [Guilin Pharmaceutical Factory No. 1, Guilin, People’s Republic of China] once a day for three days) or mefloquine-primaquine (MP) (mefloquine: 25 mg base/kg in two divided doses plus primaquine: a single adult dose of 30 mg base of primaquine phosphate [Government Pharmaceuticals, Bangkok, Thailand]). The ratio of patients receiving MA relative to MP was 2:1.

Patients were asked to come back to the designated health center every week for six weeks. The axillary temperature was measured and blood was obtained from a finger prick for
preparation of thin and thick blood films. Two drops of blood were saved after drying on filter paper. The study endpoint was the time of treatment failure defined by the presence of only asexual parasites of \textit{P. falciparum} malaria in the blood film between day 7 and day 42 of follow-up.

Recrudescences and reinfections were distinguished by parasite genotyping by a polymerase chain reaction (PCR) with blood saved on filter paper. \cite{11} Patients with recrudescence infections or reinfections were referred to the Saiyok Hospital for the confirmation of the diagnosis and re-treatment. Patients with a smear positive for \textit{P. falciparum} or for a mixed infection including \textit{P. falciparum} within 42 days of follow-up were re-treated for seven days with artesunate (total dose = 12 mg/kg).

**Statistical analysis.** Analysis for treatment failure rates and subsequent development of gametocytemia included all randomized patients who developed an outcome or completed the six-week follow-up. Because of the transient nature of gametocytemia patients who missed any of the follow-up appointments were classified as lost to follow-up in the analysis of the gametocyte carriage rate. These patients contributed person-time at risk up to the point of loss to follow-up. Some of these patients remained eligible for the calculation of the treatment efficacy if they came back for follow-up visits.

Proportions of groups were compared by a chi-square test with Yates' correction or by Fisher's exact test. Kaplan-Meier plots (taking into account the duration of follow-up for each subject) are also presented to compare the efficacy and the gametocyte carrier rates between the two treatment groups.

**RESULTS**

Between July 1999 and June 2001, 556 patients entered the study: 320 in the MA group and 236 in the MP group. There were 198 (35.6%) children less than 15 years of age (118 [36.8%] in the MA group and 80 [33.8%] in the MP group; \(P = 0.8\)). The male to female ratio was approximately 2:1 in both groups. Most cases entered the study between July and October. Overall, 141 patients (82 [25.6%] in the MA group and 59 [25%] in the MP group) did not return for follow-up. They were excluded from further analysis. However, the demographic data and geographic distribution of patients lost to follow-up within the study area were similar to those included in this analysis.

Fifteen patients in the MA group (6.3%) and 26 patients in the MP group (14.7%) had blood films positive for \textit{P. falciparum} after treatment (\(P = 0.004\)). The PCR analyses showed that seven (46.7%) of 15 patients in the MA group and five (19.2%) of 26 patients in the MP group who had blood films positive for \textit{P. falciparum} after treatment were reinfected. The rate of recrudescence over the six-week follow-up period was lower in patients in the MA group than in those in the MP group (2.5% versus 8.9%; relative risk [RR] = 0.27, 95% confidence interval [CI] = 0.11–0.62, \(P = 0.002\)). Reinfection was similar in both treatment groups (RR = 0.94, 95% CI = 0.30–2.92, \(P = 0.9\)). A summary of the treatment outcomes is shown in Table 1. Co-infection with \textit{P. vivax} was detected in eight (1.7%) patients between the third and sixth weeks of follow-up (six patients in the MA group and two patients in the MP group; \(P = 0.47\)). A Kaplan-Meier plot of the probability for recrudescence infection when compared between the two groups is shown in Figure 1.

**Subsequent gametocytemia.** Gametocytes were detected by thick blood film parasite counts in 42 (7.6%) patients on presentation (25 [7.8%] patients in the MA group and 17 [7.2%] patients in the MP group; \(P = 0.8\)). Only 24 (57.1%) of these patients came back for follow-up (14 patients in the MA group and 10 patients in the MP group). Four of 10 patients in the MP group had gametocytemia during follow-up (three of four had patent gametocytemia at the second week of follow-up). In the MA group, one patient had patent gametocytemia at the first week of follow-up and three patients had recurrent gametocytemia at the third and fifth weeks of follow-up.

Fifteen patients had gametocytemia during the 42 days of follow-up (5 patients in the MA group and 10 patients in the MP group; \(P = 0.05\)). Two-thirds of the positive follow-up slides for gametocytes were recorded by the second week (33.3% at the first week, 33.3% at the second week, 6.7% at the third week, 6.7% at the fourth week, and 20% at fifth

![FIGURE 1. Kaplan-Meier plots comparing the efficacy of mefloquine-artesunate (solid line) versus mefloquine-primaquine (dotted line) treatment for \textit{Plasmodium falciparum} malaria.](image)
The overall gametocyte carrier rate was 1.6% after treatment in the MA group and 4.2% after treatment in the MP group (RR = 0.37, 95% CI = 0.12–1.06). Subsequent gametocytemia was significantly associated with gametocytes on presentation (RR = 14.9, 95% CI = 5.9–37.6, P < 0.001) and the results of treatment. All subsequent gametocytemia developed in patients who had recrudescent infections (12 patients) or reinfections (3 patients) of *P. falciparum* malaria. All subsequent gametocytemias were detected on the day of treatment failure. The relative risks (95% CI) of developing gametocytemia were 49.8 (14.4–171.9) and 39.8 (95% CI = 9.1–173.4) in patients with recrudescence and in patients with reinfection compared with patients who were cured (P < 0.001). A Kaplan-Meier plot of the probability for subsequent gametocyte development compared between the study groups is shown in Figure 2.

**DISCUSSION**

This study confirmed that there has been a significant increase in the level of mefloquine resistance in Western Thailand for at least two years. The use of a high dose of mefloquine (25 mg base/kg) was associated with a better therapeutic response than has been reported previously, but artemesunate (three-day regimen) plus mefloquine gave the best cure rates. The carriage of gametocytes is a necessary requirement for transmission of the infection. The gametocyte carriage rate is high after monotherapy with high-dose mefloquine. Therefore, the combination of a single dose of primaquine is widely used to interrupt transmission of *P. falciparum* malaria in Thailand. However, the efficacy of this controlled intervention, which has been used for many years, has never been evaluated in controlled clinical trials. This is the first study to evaluate and compare the efficacy of primaquine and artesunate in the reduction of subsequent carriage of gametocytes. Furthermore, differentiation of reinflection from recrudescence was possible in this study by parasite genotyping by a PCR with blood saved on filter paper. The major limitation of this study was a high rate of loss to follow-up because it was difficult to achieve adequate follow-up of a large number of cases. The reasons for failure to return for outpatient follow-up are not known. However, the proportion of patients who were lost to follow-up in the two treatment groups and their demographic data were similar to those who were included in the analysis. Therefore, conclusions based on this series are probably valid for the overall population.

The results of this study showed a high rate of gametocytemia on presentation, and thus a high transmission potential of *P. falciparum* malaria in this area. Artemesunate and mefloquine do not kill mature gametocytes of *P. falciparum*. They prevent subsequent gametocyte development by their activity against precursors of this sexual stage, i.e., asexual stage parasites and early sexual stages of the parasites. Subsequent gametocytemia in patients treated with any of these antimalarial drugs is therefore directly related to the efficacy of the antimalarial treatments against asexual forms of *P. falciparum*. The purpose of the combination treatment with primaquine was to eradicate the mature gametocytes. However, persistent gametocytemia and subsequent gametocytemia in patients who had gametocytemia on presentation was similar in the two treatment groups. Treatment with mefloquine-artesunate did not significantly reduce subsequent carriage of gametocytes. The number of patient entered the study and the number of patient who developed subsequent gametocytemia were probably too small to compare an efficacy of these two treatment regimens for this outcome.

The results of this study clearly showed that recrudescent infection or reinfection were the most important factors associated with subsequent gametocytemia. It is this increased propensity of recrudescent infections to produce gametocytes that drives drug resistance. This study suggests that the rapid eradication of asexual forms of *P. falciparum* by effective antimalarial treatment was the most effective means of preventing subsequent gametocytemia. Primaquine was not effective in the eradication of gametocytes both on presentation and in the prevention of subsequent gametocytemia when compared with the artesunate therapy.

**FIGURE 2.** Kaplan-Meier plots comparing the probability of subsequent *Plasmodium falciparum* gametocytemia between patients with different treatment outcomes. Solid line = cure; dotted line = reinfection; solid/dotted line = recrudescence.
REFERENCES


