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Original article

Efficacy of a 3-day artesunate-mefloquine combination in the treatment of uncomplicated falciparum malaria in Kanchanaburi province of Thailand

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Background: In Kanchanaburi province located on the Thai-Myanmar border, *Plasmodium falciparum* parasites have developed significant resistance to commonly-used anti-malarials. For use against falciparum malaria, 2-day artesunate-mefloquine combination (MAS2) has recently been replaced by a 3-day artesunate-mefloquine combination (MAS3) that is an artemisinin-based combination therapy regimen recommended by the WHO.

Objective: Investigate the efficacy and safety of MAS3 in the treatment of uncomplicated falciparum malaria in patients of Kanchaburi province.

Methods: The study was conducted at Bongtee sub-district, Sai Yok district, Kanchanaburi province between June and November 2009. Fifty-one uncomplicated falciparum malaria patients were enrolled. Inclusion, exclusion and study method followed the WHO protocol for assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. Patients received a MAS3 and were followed for 42 days.

Results: All patients clinically recovered, but four patients were again parasitaemic on day 21, (1 patient) 28 (2 patients) and 42 (1 patient), respectively. Molecular analyses suggested that all recurrences were caused by recrudescence. There were no severe adverse events, but complaints of headache, gastrointestinal upset, nausea, and vomiting. Delay in parasite clearance was found. Proportion of parasite clearance on day 1, 2, 3 and 7 were 17.7%, 62.7%, 80.4%, and 100%, respectively.

Conclusion: MAS3 is comparable to MAS2, and meet the WHO efficacy criteria for use against falciparum malaria, but the effect on parasite clearance was inferior to that of MAS2. Close monitoring evaluation is required.

Keywords: Artesunate-mefloquine combination, efficacy study, falciparum malaria, Kanchanaburi province

In Thailand, malaria remains an important health problem, but more specifically in Kanchanaburi province, which borders Myanmar. The annual parasite incidence/1,000 populations (API) in Kanchanaburi was 1.28 in 2008 [1]. The province geography is hilly and forested, with many Anopheles mosquito breeding places. There are many illegal migrants at the border, thus causing increased reserves of malarial parasites and transmission. Some people at the border do not receive accurate diagnosis and proper treatment for malaria. This is an important contributor to antimalarial drug resistance in this region.

Plasmodium falciparum parasites in this province have developed significant resistance to commonly used antimalarials [2, 3]. The treatment of uncomplicated *P. falciparum* malaria in Thailand has been modified several times during the past 30 years

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to counter the rapid emergence and spread of drug resistance [4, 5]. A 2-day combination of artesunate-mefloquine, MAS2 (artesunate 12 mg/kg divided into two doses to be given on day 0 and 1; maximum 600 mg, mefloquine 15 mg/kg, maximum 750 mg single dose on day 0) and 0.6 mg primaquine, maximum 30 mg single dose on day 1, was introduced in Kanchanaburi province in 2002. In 2005, the dosage of mefloquine in the MAS2 regimen increased to 25 mg/kg, the same dosage as the treatment regimen of the rest of the country (maximum 1250 mg divided into two doses given on day 0 at 6 hrs interval). In 2008, Thailand has replaced MAS2 by a 3-day artesunate-mefloquine combination (MAS3) which is one of the artemisinin-based combination therapy regimens currently recommended by the WHO [6]. The total dosage is the same as the MAS2, but artesunate is divided to be given as 4 mg/kg daily on day 0, 1 and 2, to ensure that artesunate covered two parasites asexual life-cycles, thereby reducing the parasite biomass exposed to mefloquine alone [7].

In this study, we investigated the efficacy and safety of MAS3 in the treatment of uncomplicated falciparum malaria patients in Kanchanaburi province.

Materials and methods

A descriptive, efficacy, safety, and tolerability monitoring of MAS3 in the treatment of uncomplicated falciparum malaria was conducted between June and November 2009 in Bongtee subdistrict of Saiyok district, Kanchanaburi province located along the Bilauktaung Range (also known as Tenasserim or Tanaosi) bordered to Myanmar (**Fig. 1**). This study was approved by the Ethics Committee of the Department of Disease Control, Ministry of Public Health of Thailand.

Fig. 1 Map of Kanchanaburi province located at the Thai-Myanmar border.

Study area and subject

In Bongtee, there are about 2,000 inhabitants, including Thai, Mon, Karen and Myanmese. Their main occupations are gardeners, farmers, woodcutters and laborers. The climate is typically tropical with a rainy season extending from May-June to November. Malaria cases occur throughout the year, but most cases occur during the rainy season. The API of malaria in Saiyok District in 2009 was 17.3/1,000 population. Bongtee subdistrict had the highest API of 126.1/1,000 population. *Plasmodium falciparum* and *Plasmodium vivax* are present in roughly equal proportion.

Subjects who presented to the malaria clinic in Bongtee subdistrict with uncomplicated mono-infection by *P. falciparum* were enrolled. Inclusion criteria were 1) uncomplicated *P. falciparum* infection with parasitaemia between 1,000 and 100,000 parasites/μL of blood as determined by counting parasites/200 white blood cells (WBC) in Giemsa-stained thick blood films and multiplying by standard estimates of WBC counts, 6,000 WBC/μL, 2) age ≥6 months, 3) axillary temperature ≥37.5°C or a history of fever in the past 24 hours, 4) informed consent by patient or parent/guardian (in the case of children), 5) ability to come for the stipulated follow-up visits, and 6) negative urine pregnancy test for women. Exclusion criteria were 1) any sign of severe or complicated malaria, according to WHO criteria [8], and 2) breastfeeding.

Anti-malarial therapy and follow-up

Subjects with uncomplicated *falciparum* malaria received a total dose of 12 mg/kg artesunate (Guilin Pharmaceutical, Guangxi, China; 50 mg tablets) in three daily doses (4 mg/kg/day on day 0, 1, and 2, maximum 600 mg) and a total dose of 25 mg/kg mefloquine (Atlantic Laboratories, Bangkok, Thailand; 250 mg tablets) in two doses (15 mg/kg on day 0 and 10 mg/kg on day 1, maximum 1250 mg). Primaquine 0.6 mg/kg was given on day 2 (maximum 30 mg). All doses were directly observed. If vomiting occurred within 30 minutes of drug administration, the full dose was repeated. However, if it occurred 30-60 minutes, half the dosage was given again. Blood for malaria thick film examination and for parasite clearance time (PCT) test were collected from patients on days 0, 1, 2, 3, 7, 14, 21, 28, 35, and 42, and at any time when the subject reported to the clinic with fever or other symptoms suggestive of malaria. Two microscopists read all blood smears; a third reference microscopist resolved any discrepancies. Patients with recurrent *P. falciparum* parasitaemias received 7-day quinine-doxycycline combination therapy (oral quinine sulfate, 10 mg salt/kg three times daily, and doxycycline 200 mg daily in two divided doses).

Parasite genotyping

For initial infections and recurrences with *P. falciparum*, DNA isolation, and characterization of allelic polymorphisms in the genes encoding Merozoite Surface Protein 1(MSP1), Merozoite Surface Protein 2 (MSP2), and Glutamate-Rich Protien (GLURP) by PCR were performed as described [9]. A new infection is a subsequent occurring parasitaemia in which all the alleles in parasites from the post-treatment sample are different from those in the admission samples, for one or more loci tested. In a recrudescence, at least one allele at each locus is common to both paired samples [10].

Statistical analysis

Treatment efficacy was determined based on WHO classification of treatment outcome [13] as follows: 1) Early treatment failure (ETF): danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia; or parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; or parasitaemia on day 3 with axillary temperature ≥37.5°C; or parasitaemia on day 3 ≥25% of count on day 0, 2) Late clinical failure (LCF); danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 42 in patients who did not previously meet any of the criteria of ETF; or presence of parasitaemia on any day between day 4 and day 42 with axillary temperature ≥37.5°C in patients who did not previously meet any of the criteria of ETF, 3) Late parasitological failure (LPF); presence of parasitaemia on any day between day 7 and day 42 and axillary temperature < 37.5°C in patients who did not previously meet any of the criteria of ETF or LCF, and 4) Adequate clinical and parasitological response (ACPR); absence of parasitaemia on day 42, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of ETF, LCF or LPF.

Recurrence denoted clinical recurrence of malaria after the initial clearance of parasite from circulation. Parasite reappearance after day 3 was interpreted as either true recrudescence or a new infection by

genotyping analysis. In the case of subjects recurrent with *P. falciparum* malaria, the results of both per protocol and Kaplan-Meier analysis are reported.

In the per protocol analysis, the proportion of treatment failures was calculated by dividing the total number of subjects with recurrent parasitaemias (with PCR correction for re-infection) by the number of subjects who either suffered recurrent parasitaemia or completed the full 42-day follow-up period.

In the Kaplan-Meier analysis, subjects were censored from the point at which they 1) were lost to follow-up or 2) acquired a *P. vivax* infection. Subjects were considered to have cleared parasitaemia if there were at least two sequential negative smears. The day on which the first such negative smear was observed was defined as the day of clearance. Since smears were not taken on days 4 to 6, subjects with a reported clearance day 7 may actually have cleared their parasitaemia on any day between day 4 and 7.

Results

Demographic and clinical characteristics of the study patients

The efficacy of a MAS3 in the treatment of uncomplicated *falciparum* malaria was monitored. The first patient was enrolled in June 2009, and the study was completed in November 2009. Fifty-one patients completed the study.

The median age was 30 years, minimum and maximum ages were 6 and 80 years. Patients with age greater than five years were enrolled in the study. Forty-one patients (82%) were male. The geometric mean parasite on the day of enrolment (day 0) was 14,362 parasite/μL (95% confidence interval 9,521-21,667). Gametocyte carriage was found in 3 patients (5.9%). Fifty patients (98%) had fever on day 0.

Treatment efficacy

According to the WHO assessment criteria, one LCF and 3 LPF were found in this study. All were treated successfully with 7-day quinine-doxycycline combination, which is the second line treatment of uncomplicated falciparum malaria in Thailand. There was no ETF patient.

Figure 2 shows two examples of Kaplan Meier survival efficacy analysis of uncomplicated falciparum malaria patient. The Kaplan Meier estimate of the PCR corrected 42-day efficacy rate was 92.2% (95% CI 81.1-97.8).

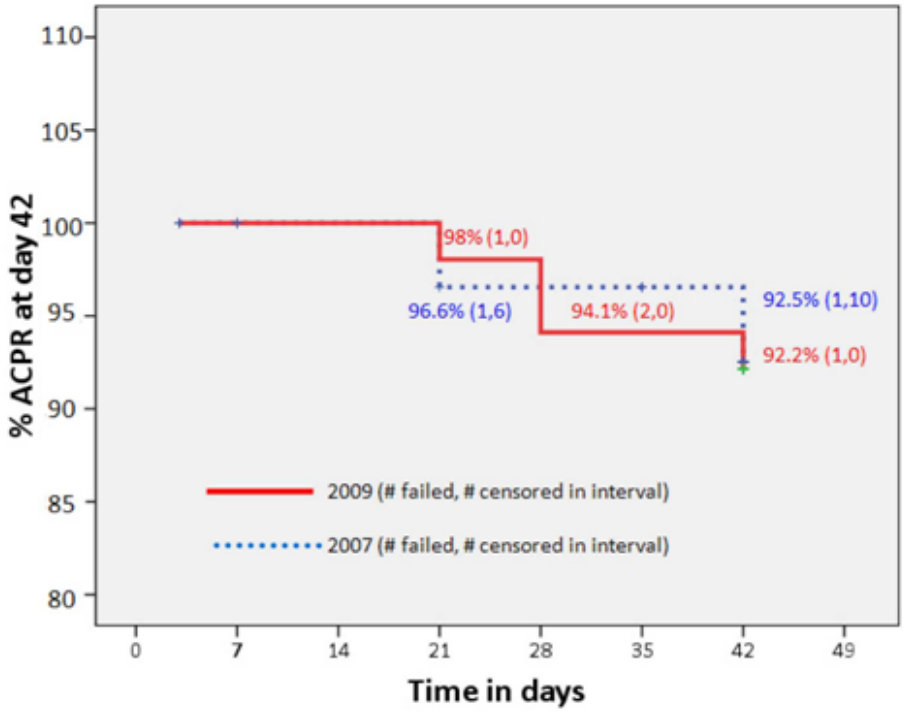

Fig. 2 Kaplan Meier survival efficacy analysis of uncomplicated falciparum malaria patient in 2007 and 2009. ACPR: Adequate clinical and parasitological response.

Parasite clearance

On day 1, nine (17.7%) out of the 51 enrolled patients no longer had any malaria parasites in their blood. The geometric mean parasite densities in the remaining 42 participants were drastically reduced to 419 parasites/μL (95% CI 221-795). The clearance rate dramatically increased to 62.7% and 80.4% on day 2 and day 3 until total clearance was achieved in the remaining 10 patients on day 7.

Parasite clearance time was determined from the spreadsheet data [11]. Parasitaemia completely cleared in nine patients within 24 hours, 23 within 48 hours, and nine within 72 hours, while 10 patients were cleared in 96 hours resulting in the average time to parasite clearance of 57.4 hours.

Temperature clearance profile

One out of the 51 patients had temperatures below 37.5°C on enrolment (day 0), but had history of fever on the previous 24 hours. The mean (±SD) temperature of the 50 patients with temperatures ≥ 37.5°C was 38.5°C±0.76°C. The temperature dropped to a mean value of 37.6°C±0.41°C and 37.2°C±0.23°C on day 1 and day 2, respectively.

Anti-gametocyte activity

The number of patients who carried gametocyte was three (5.9%), three (5.9%), one (2%), and one (2%) on day 0, 1, 2, 3 and 7, respectively. One patient carried gametocyte until day 7. In this patient, the gametocyte densities on day 0, 1, 2, 3, and 7 were 660, 330, 180, 96, and 84 parasites/μL, respectively. Two patients carried gametocytes on day 0, but they were cleared by day 1. In these two patients, the gametocyte densities were 60 and 90 parasites/μL. There were two patients with agametocytaemia on day 0, but the gametocyte densities were 108 and 36 gametocytes/μL on day 1. There was one recrudescent patient with sexual and asexual parasitaemia on day 28. There was no gametocyte found on day 0.

Safety and tolerability

No serious adverse event (SAE) was reported during the study. Many adverse events (AE), most likely related to the underlying malaria disease such as headache, muscle pain, and anorexia, were reported. These symptoms disappeared by day 2 or day 3 after the treatment. Two symptoms were reported more on day 1 and day 2. On day 0, 26 patients (52%) reported dizziness and 11 (22%) sleeplessness. More patients reported the dizziness (29 patients, 58%) and sleeplessness (28 patients, 56%) on day 2 and day 3.

Discussion

The therapeutic efficacy study of MAS3 in Kanchanaburi province showed both effective and well tolerated in the treatment of acute uncomplicated falciparum malaria. There was no difference (p=0.936) in cure rate by the log rank test for homogeneity between this study (92.2%) and the year 2007 study of MAS2 (92.5%) as shown in **Fig. 2**. The parasitological cure rate in 2007 study was 97% on day 1 and 100% on day 2.

In this study, the geometric mean parasite density was drastically reduced but only 17.7% of the patients were aparasitaemic on day 1. On day 2 and day 3, the percentages of patients' aparasitaemic were 62.7% and 80.4%, respectively. The parasites were cleared completely during day 4 - day 7.

The treatment regimens in Kanchanaburi province in 2003 was the two-day regimen of MAS2 (15 mg/kg M and 12 mg/kg AS). The national malaria control program of Thailand replaced it by MAS2 (25 mg/kg and 12 mg/kg AS) in 2005 and to the 3-day regimen of MAS3 (M 25mg/kg and AS 12 mg/kg) in 2008.

The efficacies of the treatment regimens were monitored during 2003-2009 in the same population group in Sai Yok district. **Figure 3** shows the percentage of patients' aparasitaemic on day 2 and day 3. In 2003, the patients' aparasitaemic were 87% and 90.7% on day 2 and day 3. When the dosage of mefloquine increased to 25 mg/kg in the 2005 and 2007 regimens, the patients' aparasitaemic on day 2 increased to 100%.

The national malaria control program decided to replace the MAS2 regimen by the MAS3 regimen as recommended by the World Health Organization based on the theory that prolonged treatment with artesunate from two to three days will delay the development of resistance to artesunate. However, the present results showed the prolonged parasite clearance time. The patients' aparasitaemic in 2009 were 62.7% and 80.4% on day 2 and day 3, respectively. The difference between the MAS2 and MAS3 regimens was that the 12 mg/kg artesunate in the MAS2 was divided to be given 6 mg/kg daily for 2 days but for the MAS3, artesunate was divided to be given 4 mg/kg daily for

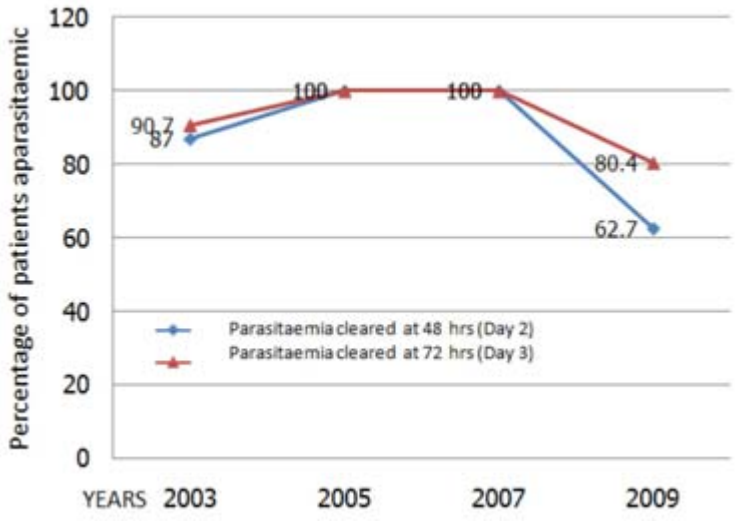

Fig. 3 Percentage of patients' aparasitaemic on day 2 and day 3 following the treatment of uncomplicated falciparum malaria patients in Kanchanaburi province during the years 2003-2009. Data of the years 2003, 2005 and 2007 were obtained from the efficacy results monitored by the national malaria control program of Thailand

three days. The lower dosage of artesunate given on day 0 in the MAS3 regimen may be the explanation of the delay in parasite clearance.

In this study, we showed the rapid clinical response by a drop in temperature to normal values (*viz.* below 37.5°C) on day 2. Apart from the rapid clearance of asexual forms of *P. falciparum*, MAS therapy was also beneficial in inducing significant reduction in gametocyte rate and density [6]. In this study, only three patients presented with gametocytaemia on day 0, indicating patients who sought treatment early and were diagnosed early. However, one patient maintained gametocytaemia until day 7. On the enrolment, he had high gametocytaemia (660 gametocytes/μL *vs.* 7,812 ring forms/μL). The delay in gametocyte clearance might be due to the insufficient dosage of artesunate in getting rid of high-density gametocytaemia, and the delay in giving primaquine might be effective for killing gametocyte from day 1 in the MAS2 to day 2 in the MAS3 regimens. There was evidence that artemisinin derivatives may kill young gametocyte, not mature gametocyte [14].

Conclusion

This study proved the efficacy of the MAS3 in the treatment of uncomplicated falciparum malaria in Kanchanaburi province, Thailand. MAS3 is comparable to MAS2, and meet the WHO efficacy criteria for use against falciparum malaria, but the effect on parasite clearance was inferior to that of MAS2. Delay in parasite clearance is alarming and need closed monitoring and review of the appropriate dosage of the treatment regimen.

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References

1. Bureau of Vector Borne Disease. Malaria situation. Nonthaburi:Ministry of Public Health of Thailand, 2008. Available at http://www.thaivbd.org/cms.
2. Vijaykadga S, Rojanawatsirivej C, Cholpol S, Phoungmanee D, Nakavej A. *In vivo* sensitivity monitoring of mefloquine monotherapy and artesunate-mefloquine combinations for the treatment of uncomplicated falciparum malaria in Thailand in 2003. Trop Med Int Health. 2006; 11: 211-9.
3. Suputtamongkol Y, Chindarat S, Silpasakorn S, Chaikachonpatd S, Lim Kimheng, Chanthapakajee K, et al. The efficacy of combined mefloquine-artesunate versus mefloquine-primaquine on subsequent

development of *Plasmodium falciparum* gametocytemia. Am J Trop Med Hyg. 2003; 68:620-3.
4. Chareonviriyaphap T, Bangs MJ, Ratanatham S. Status of malaria in Thailand. Southeast Asian J Trop Med Public Health. 2000; 31: 225-37.
5. Rooney W. Dynamics of multi-drug resistance in *Plasmodium falciparum* in Thailand. Southeast Asian J Trop Med Public Health. 1992; 23 (Suppl 4): 131-7.
6. Agomo PU, Meremikwu MM, Watila IM, Omalu IJ, Friday AO, Oguche S, et al., Efficacy, safety and tolerability of artesunate-mefloquine in the treatment of uncomplicated *Plasmodium falciparum* malaria in four geographic zones of Nigeria. Malar J. 2008; 7: 172.
7. White NJ. Assessment of the pharmacodynamic properties of antimalarial drugs *in vivo*. Antimicrob Agents Chemother. 1997; 41:1413-22.
8. World Health Organization. Management of severe malaria: a practical handbook. Second edition. Geneva: WHO, 2000. Available at http://apps.who.int/malaria/docs/hbsm.pdf.
9. Farnert A, Arez AP, Babiker HA, Beck HP, Benito A, Bjorkman A, et al. Genotyping of *Plasmodium falciparum* infections by PCR: a comparative multicentre study. Trans R Soc Trop Med Hyg. 2001; 95:225-32.
10. World Health Organization. Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations. Geneva: WHO, 2008. Available at http://www.who.int/malaria/resistance.
11. WHO/MAL/82.988. Methods for surveillance of antimalarial drug efficacy. Geneva:WHO. Available at http://www.who.int/malaria/resistance.
12. World Health Organization. Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. WHO/HTM/RBM/2003.50. Geneva:WHO, 2003. Available at http://www.who.int/malaria/resistance.
13. World Health Organization. Susceptibility of *Plasmodium falciparum* to antimalarial drugs. Report on global monitoring 1996-2004. WHO/HTM/MAL/2005.110, Geneva:WHO. 2005. Available at http://www.who.int/malaria/resistance.
14. World Heath Organization. Guidelines for the treatment of malaria. 1st ed. Geneva, Switzerland. WHO:2006, p. 133-343.