A COMPARATIVE CLINICAL TRIAL OF COMBINATIONS OF DIHYDROARTEMISININ PLUS AZITHROMYCIN AND DIHYDROARTEMISININ PLUS MEFLOQUINE FOR TREATMENT OF MULTIDRUG RESISTANT FALCIPARUM MALARIA

S Krudsood[1], K Buchachart[1], K Chalermrut[2], C Charusabha[1], S Treeprasertsuk[3], O Haoharn[2], C Duangdee[2] and S Looareesuwan[3]

[1]Department of Tropical Hygiene; [2]Hospital for Tropical Diseases; [3]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. With the deteriorating situation of multidrug resistant falciparum malaria, a new drug or drugs in combinations are urgently needed. We conducted a study comparing a combination of dihydroartemisinin 240 mg and mefloquine 1,250 mg given over 3 days (Group 1) and a combination of dihydroartemisinin 240 mg and azithromycin 1,500 mg given over 3 days (Group 2), to determine safety, efficacy and tolerability. All of the patients stayed in a non-malaria endemic area during the study. By the third day after drug administration, most patients were free of parasites and none had serious adverse events. The cure rates at day 28 were 100% and 69.7% in Group 1 and Group 2, respectively ($p<0.01$). We conclude that a combination of dihydroartemisnin and azithromycin was safe and effective and may be another interesting regimen of the treatment of uncomplicated multidrug resistant *Plasmodium falciparum* malaria in Thailand.

INTRODUCTION

Multidrug resistant falciparum malaria is a serious problem in Thailand. Therapeutic failures with all available antimalarial drugs are well documented. Quinine and tetracycline given for seven days, now a standard regimen for multidrug resistant *Plasmodium falciparum* strains in Thailand, gave 90-98% cure rate when it was given under supervision (Looareesuwan *et al*, 1992b; 1994a). With this deteriorating situation, new drugs and drugs in combinations which are well tolerated and simple to use are urgently needed. Most recent guidelines in treating malaria strongly recommended the use of a combination of artemisinin derivatives with other antimalarial agents to avoid the emergence of resistance (World Health Organization Model List of Essential Drugs, 1999), and artemisinin combinations are considered a central component of the global Roll Back Malaria initiative as first line treatment.

Dihydroartemisinin, an artemisinin derivative, has been evaluated in clinical trials in Thailand for the treatment of falciparum malaria since 1994 (Looareesuwan *et al*, 1996b; Wilairatana *et al*, 1998). It is a potent antimalarial drug that can reduce parasitemia by 90% within 24 hours of administration (Looareesuwan S *et al*, 1996a; Klayman, 1985) and in previous studies gave an 80-92% cure rate (Looareesuwan S *et al*, 1996b; Wilairatana *et al*, 1998). All the artemisinin derivatives are metabolized rapidly to the active metabolite dihydroartemisinin (Luxemburger *et al*, 1995). This drug is easy to produce with less synthetic steps and, thus, a lower cost.

Mefloquine, a quinoline methanol, has a long half-life (2-3 weeks) and could be given in a single dose. This drug used to have a satisfactory response with a cure rate of 98%

Correspondence: Dr Srivicha Krudsood, Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand.
Tel : +66 (0) 2644-7483, Fax : +66 (0) 2644-4436
E-mail: tmsks@mahidol.ac.th

when used alone from 1983-1986 (Harinasuta *et al*, 1987; Nosten *et al*, 1987). By 1990, the efficacy at the dose level of 15 mg/kg had rapidly declined to 71% (Nosten F *et al*, 1991). Recently, artesunate followed by mefloquine has proved effective and well tolerated (Looareesuwan *et al*, 1992c; 1994b; 1996a; Nosten *et al*, 1994; Prince *et al*, 1995; 1997). Adverse effects of mefloquine are reported commonly.

Azithromycin, a relatively recent semisynthetic derivative of erythromycin posses antimalarial effects and has been used for prophylaxis and treatment of falciparum malaria (Gingras and Jansen, 1993; Kushner *et al*, 1994; Andersen *et al*, 1994a,b; 1995, Na-Bangchang *et al*, 1996; Taylor *et al*, 1999; Biswas, 2001; Noedl *et al*, 2001; Kain *et al*, 2001; Krudsood *et al*, 2000). It has pharmacokinetic and pharmacodynamic properties that allow for a simple dosing regimen with minimal side effects (White and Breman, 1998; De Mol *et al*, 1996; Peters *et al*, 1992) and can be used in children and pregnant women.

This study aimed to determine the safety, tolerability and efficacy of dihydroartemisinin when used in the combinations with azithromycin and mefloquine for the treatment of multidrug-resistant falciparum malaria, as potential alternative antimalarial regimens.

MATERIALS AND METHODS

This sequential, open-label trial, was conducted in Thailand at Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University (Bangkok, Thailand). Approval was given by the Ethics Committee of the Faculty of Tropical Medicine. Patients 15 years of age or older, and more than 39 kg in body weight, presenting with microscopically confirmed *Plasmodium falciparum* malaria were eligible for the trial if they or a responsible family member gave inform consent. The trial excluded subjects with signs and symptoms indicative of severe or complicated malaria (WHO, 2000), inability to tolerate oral medications, pregnancy or lactation, allergy or sensitivity to drugs and consumption of any antimalarial drug therapy within 2 weeks prior to admission. All patients were admitted in the hospital for 28 days or agreed to stay in non-malaria endemic areas and came for follow-up weekly to complete a 28-day follow-up in order to observe adverse effects and to exclude reinfection.

Each patient was sequentially assigned to one of two regimens: Group 1 was given dihydroartemisinin 80 mg or 4 tablets (Cotexin® Beijing Cotec New Technology Corp, Beijing Wan Hui Pharmaceutical group, China, 20 mg/tablets) together with mefloquine 10 mg/kg (2 tablets) for the first two days then followed by dihydroartemisinin 80 mg or 4 tablets together with mefloquine 5 mg/kg (1 tablets) on the third day of treatment; Group 2 was given dihydroartemisinin 80 mg or 4 tablets together with azithromycin 500 mg (2 capsules) for 3 days. Patients who vomited within one hour after drug administration were redosed.

At enrolment, a medical history was obtained, a full baseline physical examination and blood was taken for routine hematology and biochemistry. The clinical signs and symptoms were recorded daily during the first week and then weekly until patients were discharged. Oral temperature, pulse and respiratory rate were obtained every 4 hours while blood pressure was measured once daily. Routine laboratory examinations were performed on day 0, 7, 14, 21 and 28. Malaria parasite counts were examined prior to treatment and every 12 hours until negative then once daily for 28 days in hospital. Blood films were considered negative if no parasites were seen in 200 oil immersion fields. Parasite counts per μl using Giemsa stained, were determined by counting the number of asexual parasites against 200 white blood cells in thick blood films or against 1,000 red blood cells in thin blood films.

Clinical efficacy was assessed by using the 28-day cure rate and parasite and fever clearance time. The 28-day cure rate was defined as the proportion of patients who cleared asexual

parasitemia within seven days of initiation of treatment without subsequent recrudescence within 28 days and it was evaluated for only the intention-to-treat (ITT) patients. Parasite clearance time (PCT) was defined as the time from the start of treatment until the first negative blood film and remained negative for the next 24 hours. Fever clearance time (FCT) was defined as the time taken for a patient's temperature to decrease to 37.5ºC and remain there for at least next 48 hours.

Standard descriptive and statistical analysis were conducted using version 6.04 of the EpiInfo software (Centers for Disease Control, Atlanta, GA). Comparisons were made using chi-square and Student's *t*-test, Mann-Whitney U test.

RESULTS

A total of 170 patients (88 and 82 in Group 1 and Group 2, respectively) enroled into the study. One hundred and thirty-four patients completed the study. Thirty-six patients (20 patients in Group 1 and 16 patients in Group 2, respectively) were lost to follow-up after day 7, however all of them were cured before they left the hospital. Only patients who were followed for 28 days were included in calculations of drug efficacy.

Table 1 gives the baseline characteristics of the patients. There were 122 male and 48 female patients aged 15 to 72 years old participated in the study. The distribution of demographic, clinical and laboratory data did not show any statistically significant difference between the two treatment groups.

At enrolment, patients in both treatment groups showed common malaria symptoms such as headache, asthenia, fatigue, fever, dizziness, 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.

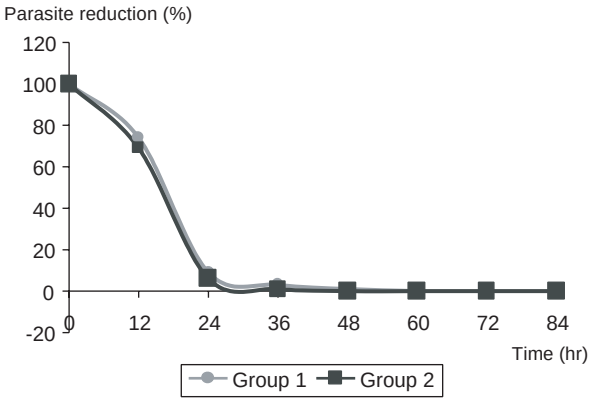

Fig 1–Means of malaria parasite reduction (%) after treatment.

All patients in this study showed a prompted response to both antimalarial regimens (Fig 1). Table 2 summarized the responses for the efficacy endpoints. The 28-day cure rate was highly efficacious in Group 1 (100%) while it was only 69.7% in Group 2 ($p<0.01$). The median of *Plasmodium falciparum* reappearance in Group 2 was 21 days (min-max = 16-30 days). In addition, there was no RII or RIII response in this study. All of the patients whose drug failed to clear parasitemia were given the rescue antimalarial chemotherapy according to the hospital's standard regimen.

Means time for parasite clearances in each treatment group was fast, however there were no statistically significant differences between the two treatment-groups [44.2±12.7 hours and 43.3±11.7 hours in Group 1 and Group 2, respectively, ($p=0.67$)]. The parasites were all cleared from peripheral blood smears within 79 hours. Similarly, median times (due to non-normal distribution) for fever clearance did not show any differences [32 hours and 30 hours in Group 1 and Group 2, respectively ($p=0.74$)].

Regarding safety, it was difficult to distinguish between symptoms of acute malaria and drug-related side effects. All signs and symptoms and parasitemia simultaneously disappeared within few days. In addition, there

Table 1
Baseline clinical and laboratory characteristics of the patients.

		Group 1 (n=88)	Group 2 (n=82)	p-value
Age (years)	Mean ± SD	25.2 ± 11.1	27.0 ± 1.9	0.23
	(min-max)	(15 - 62)	(15 - 72)	
Sex (Male:Female)	No. (% Male)	64 : 24 (72%)	58 : 24 (70%)	0.74
Duration of fever before admission	Mean ± SD	5.3 ± 6.1	6.0 ± 7.6	0.56
	(min-max)	(1 - 30)	(1 - 60)	
History of malarial infection	No. (%)	42 (47.7)	31 (39.2)	0.27
Hepatomegaly	No. (%)	6 (6.8)	8 (9.8)	0.47
Splenomegaly	No. (%)	7 (8.0)	6 (7.3)	0.89
Initial parasite count	Geometric mean	5,754	9,772	0.08
	(min-max)	44 - 375,000	35 - 217,980	
Laboratory data				
Hematocrit (%)	Mean ± SD	35.6 ± 6.6	35.1 ± 7.0	0.67
	(min-max)	(20 - 51)	(18 - 49)	
WBC count (per µl)	Mean ± SD	5.7 ± 2.4	5.2 ± 1.8	0.18
	(min-max)	(2.2 - 17.5)	(2.1 - 10.3)	
Total bilirubin (mg/dl)	Mean ± SD	1.5 ± 1.4	1.5 ± 1.3	0.66
	(min-max)	(0.36 - 8.3)	(0.30 - 9.56)	
Direct bilirubin (mg/dl)	Mean ± SD	0.5 ± 0.8	0.5 ± 0.6	0.51
	(min-max)	(0.10 - 4.14)	(0.09 - 3.80)	
SGOT (U/l)	Median	34.0	32.5	0.93
	(min-max)	(15 - 140)	(12 - 252)	
SGPT (U/l)	Median	28.0	37.0	0.24
	(min-max)	(10 - 218)	(8 - 492)	
Albumin (g/dl)	Mean ± SD	3.9 ± 0.6	4.0 ± 0.6	0.56
	(min-max)	(2.5 - 5.0)	(2.4 - 4.9)	
BUN (mg/dl)	Mean ± SD	15.0 ± 9.0	15.0 ± 6.4	0.98
	(min-max)	(4.0 - 55.0)	(5.0 - 44.0)	
Cr (mg/dl)	Mean ± SD	0.9 ± 0.4	0.9 ± 0.2	0.86
	(min-max)	(0.56 - 2.27)	(0.65 - 1.50)	
G6PD deficiency (Male:Female)	No. (%)	5 : 79 (6.1)	8 : 70 (10.2)	0.32

was no serious adverse event reported during the study.

DISCUSSION

With the deteriorating situation of multidrug resistant falciparum in Thailand (Looareesuwan *et al*, 1992a), attempts have been made to delay the advent of resistance by the use of certain drug combinations. Regarding this problem, the rationale for using a single compound warrants change. The mechanism of action of different drugs varies, and they can act in different biosynthetic pathways of the plasmodium parasite. Therefore, drug combinations may also prevent the development of resistance.

Similar to our previous study (3-day combination of artesunate with azithromycin) (Krudsood *et al*, 2000), a 3-day combination of dihydroartemisinin with azithromycin gave an approximately 70% cure rate. Moreover, this 3-day combination gave a better cure rate when compared with the 2-day azithromycin combination with artemether (14.8% cure rate) (Na-Bangchang *et al*, 1996) which could be related

Table 2
Parasitological and clinical outcomes.

		Group 1 (n=88)	Group 2 (n=82)	p-value
No. of patients with 28-day follow-up	No. (%)	68 (77.3)	66 (80.5)	
No. of patients drop out	No. (%)	20 (22.7)	16 (19.5)	
Fever clearance time (FCT) (hours)	Median	32	30	0.74
	(min-max)	(4-180)	(4-284)	
Parasite clearance time (PCT) (hours)	Mean ± SD	44.2 ± 12.7	43.3 ± 11.7	0.67
	(min-max)	(6-79)	(12-68)	
Cure rate at day 28	No. (%)	68 (100)	46 (69.7)	< 0.01
No. of recrudescense	No. (%)	0 (0)	20 (30.3)	< 0.01
Recrudescense day	Median	NA	21	
	(min-max)	NA	16-30	

to the longer duration and higher dosage of both azithromycin and artemisinin derivatives administration. In contrast, a combination of dihydroartemisinin with mefloquine gave highly efficacious outcome (100% at 28-day cure rate). However, the percents of parasite reduction of both regimens did not show any difference. This may due to the effect of dihydroartemisinin. In addition, azithromycin may give an additive effect. Pharmacokinetic data are needed for measurement of these compounds in plasma or serum.

Like a previous study (Wilairatana *et al*, 1998) most of the patients in this study improved clinically and were parasite negative on the blood smear by the third day of treatment in both combination regimens. These combinations may serve as alternative regimens for treatment of uncomplicated falciparum malaria, especially the combination of dihydroartemisinin with azithromycin which is useful in pregnancy and in children in whom tetracycline and doxycycline are contraindicated.

Secondly, many febrile illnesses in endemic areas mimic malaria, and confirmatory parasitologic diagnosis is not often available, reliable, or prompt, particularly in rural zones. Parasitemic patients can also have other illnesses, complicating matters even more. Azithromycin which posses antibiotic activity against community-acquired respiratory tract, skin and soft tissue bacterial infections, and against sexually transmitted disease could have dual benefits. Nevertheless, the relatively high cost of this combination may limit the usefulness of azithromycin in malaria therapy.

In conclusion, a highly efficacious combination of dihydroartemisinin with mefloquine may serve as an alternative regimen for the treatment of uncomplicated falciparum malaria in areas where multidrug resistant *Plasmodium falciparum* malaria is prevalent. On the other hand, the combination of dihydroartemisinin with azithromycin which gave the lower cure rate, may be useful in children and pregnant women and areas where parasitologic diagnosis is not available. Further study is required in order to enhance its clinical efficacy.

ACKNOWLEDGEMENTS

We thank the staff at the Hospital for Tropical Diseases for their help. This study was supported by a Mahidol University Research Grant.

REFERENCES

Andersen SL, Ager AL, McGreevy P, *et al*. Activity of azithromycin as a blood schizonticide against rodent and human plasmodia in vivo. *Am J Trop*

Med Hyg 1994a; 52: 159-60.

Andersen SL, Ager AL, McGreevy P, Schuster BG, Ellis W, Berman J. Efficacy of azithromycin as a casual prophylactic agent against murine malaria. *Antimicrob Agents Chemother* 1994b; 38: 1862-3.

Andersen SL, Berman J, Kuschner R, *et al*. Prophylaxis of *Plasmodium falciparum* malaria with azithromycin administered to volunteers. *Ann Intern Med* 1995; 123: 771-3.

Biswas S. *In-vitro* antimalarial activity of azithromycin against chloroquine sensitive and chloroquine resistant *Plasmodium falciparum*. *J Postgrad Med* 2001; 47: 240-3.

De Mol P, Alimenti A, Franchart G, Waelbroeck A, Lery J. Azithromycin versus erythromycin in the treatment of lower respiratory tract infections in children. *ICMAS* 1996: 76-7.

Gingras BA, Jansen JB. Antimalarial activity of azithromycin and erythromycin against *Plasmodium berghei*. *Am J Trop Med Hyg* 1993; 49: 101-5.

Harinasuta T, Bunnag D, Vanijanonta S, *et al*. Mefloquine, sulfadoxine and pyrimethamine in the treatment of symptomatic falciparum malaria: a double-blind trial for determining the most effective dose. *Bull WHO* 1987; 63: 363-7.

Kain KC, Shanks GD, Keystone JS. Malaria chemoprophylaxis in the age of drug resistant. I. Currently recommended drug regimens. *Clin Infect Dis* 2001; 33: 226-34.

Klayman DL. Qinghaosu (artemisinin): an antimalarial drug from China. *Science* 1985; 228: 1049-55.

Krudsood S, Silachamroon U, Wilairatana P, *et al*. A randomized clinical trial of combinations of artesunate and azithromycin for treatment of uncomplicated *Plasmodium falciparum* malaria in Thailand. *Southeast Asian J Trop Med Public Health* 2000; 31: 801-7.

Kushner RA, Heppner DG, Andersen SL, *et al*. Azithromycin prophylaxis against a chloroquine-resistant strain of *Plasmodium falciparum*. *Lancet* 1994; 343: 1396-7.

Looareesuwan S, Harinasuta T, Chongsuphajaisiddhi T. Drug resistant malaria with special reference to Thailand. *Southeast Asian J Trop Med Public Health* 1992a; 23: 621-34.

Looareesuwan S, Wilairatana P, Vanijanonta S, *et al*. Efficacy of quinine-tetracycline for acute uncomplicated falciparum malaria in Thailand. *Lancet* 1992b; 339: 69.

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* 1992c; 74: 794-9.

Looareesuwan S, Vanijanonta S, Viravan C, *et al*. Randomized trial of mefloquine-tetracycline and quinine-tetracycline for acute uncomplicated falciparum malaria. *Acta Trop* 1994a; 57: 47-53.

Looareesuwan S, Vanijanonta S, Viravan C, Wilairatana P, Charoenlarp P, Andrial M. Randomized trial of mefloquine alone, and artesunate followed by mefloquine for the treatment of acute uncomplicated falciparum malaria. *Ann Trop Med Parasitol* 1994b; 88: 131-6.

Looareesuwan S, Viravan C, Vanijanonta S, Wilairatana P, Pittisuthithum P, Andrial M. Comparative clinical trial of artesunate followed by mefloquine in the treatment of uncomplicated falciparum malaria: two and three-day regimens. *Am J Trop Med Hyg* 1996a; 54: 210-3.

Looareesuwan S, Wilairatana P, Vanijanonta S, *et al*. Treatment of acute uncomplicated falciparum malaria with oral dihydroartemisinin. *Ann Trop Med Parasitol* 1996b; 90: 21-8.

Luxemburger C, Nosten F, White NJ, *et al*. Oral artesunate in the treatment of uncomplicated hyperparastemic falciparum malaria. *Am J Trop Med Hyg* 1995; 53: 522-25.

Na-Bangchang K, Kanda T, Tipawangso P, *et al*. Activity of artemether-azithromycin versus artemether-doxycycline in the treatment of multiple drug resistant falciparum malaria. *Southeast Asian J Trop Med Public Heath* 1996; 27: 522-5.

Noedl H, Wernsdorfer WH, Krudsood S, *et al*. Antimalarial activity of azithromycin, artemisinin and dihydroartemisinin in fresh isolates of *Plasmodium falciparum* in Thailand. *Acta Trop* 2001; 80: 39-44.

Nosten F, Imvithaya S, Vincenti M, *et al*. Malaria on the Thai-Burmese border: treatment of 5,192 patients with mefloquine-sulfadoxine-pyrimethamine. *Bull WHO* 1987; 65: 891-6.

Nosten F, Luxemburger C, ter Kuile F, *et al*. Optimum artesunate-mefloquine combination for the treatment of multidrug resistant *Plasmodium falciparum* malaria. *J Infect Dis* 1994; 170: 971-7.

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

Peters DH, Friedel A, McTavish D. Azithromycin: a review of its antimicrobial activity, pharmocokinetic properties and clinical efficacy. *Drugs* 1992; 44: 750-9.

Prince RN, Nosten F, Luxemburger C, *et al*. Artesunate versus artemether in combination with mefloquine for the treatment of multidrug resistant falciparum malaria. *Trans R Soc Trop Med Hyg* 1995; 89: 523-7.

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

Taylor WR, Richie TL, Fryauff DJ, *et al*. Malaria prophylaxis using azithromycin: a double blind, placebo-controlled trial in Irian Jaya, Indonesia. *Clin Infect Dis* 1999; 28: 74-81.

White NJ, Breman JG. Malaria and other diseases caused by red blood cell parasties. In: Fauci AS, Braunwald E, Isselbacher KJ, *et al*, eds. Harrison's Principles of Internal Medicine. 14th ed. USA: McGraw-Hill. 1998: 1180-9.

Wilairatana P, Chantavanich P, Singhasivanon P, *et al*. A comparison of three different dihydroartemisinin formulations for the treatment of acute uncomplicated falciparum malaria in Thailand. *Int J Parasitol* 1998; 28: 1213-18.

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