

CLINICAL TRIAL OF ARTESUNATE AND ARTEMETHER ON MULTIDRUG RESISTANT FALCIPARUM MALARIA IN THAILAND. A PRELIMINARY REPORT

Danai Bunnag, Chaisin Viravan, Sornchai Looareesuwan, Juntra Karbwang and
Tranakchit Harinasuta.

Department of Clinical Tropical Medicine, Bangkok Hospital for Tropical Diseases,
Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Abstract. A 5-day course of oral artesunate at total doses of 1200, 600, 650 mg and intramuscular artemether 480 mg proved effective (90-100% cured) in the treatment of multidrug resistant falciparum malaria in Thailand. Shorter courses yielded high recrudescence rates. The fever clearance and parasite clearance times were short. The side effects were mild and transient including occasional abnormal electrocardiograms and pain at the injection site. Slight reduction of neutrophil leucocytes and reticulocytes was observed. Further studies of artesunate and artemether should be carried out to find the optimum dosage regimen and to clarify the hematological effects.

INTRODUCTION

The treatment of falciparum malaria is a major medical problem in Thailand since the parasite is highly resistant to chloroquine and sulfadoxine-pyrimethamine (Harinasuta *et al*, 1965; Reacher *et al*, 1981), and there is increasing resistance to alternative antimalarials such as quinine, mefloquine and mefloquine/sulfadoxine/pyrimethamine (Bunnag *et al*, 1986). Consequently, there is an urgent need to develop new effective drugs. Artesunate and artemether are two such drugs currently being tested.

Artesunate and artemether are the hemisuccinate and methyl ether derivatives, respectively, of Qinghaosu (artemisinin), the active antimalarial compound extracted from a medicinal herb "Qinghao" (*Artemisia annua L.*). Its use dates at least from 168 BC and it is included in the "Recipes of treatment" of 52 diseases found in the Mawangdui Han Tomb, Changsa, Hunan, China (Klayman, 1985). The early experiences of the treatment of malaria with artemisinin and its derivatives were reviewed by the Qinghaosu Antimalarial Coordinating Research Group in 1979. The drugs

were found to be effective against *P. vivax* and both chloroquine-sensitive and chloroquine-resistant *P. falciparum* (Jiang *et al*, 1982; Li *et al*, 1984). Until recently, they have not been generally available outside China. Most of the clinical trials were performed in China and in a few selected groups of subjects in Burma (Myint and Shwe, 1987; Shwe *et al*, 1988). More formal clinical trials are needed to evaluate the efficacy and safety of artemisinin analogues. This report describes the first clinical trial of these preparations carried out in Thailand.

MATERIALS AND METHODS

The study was carried out in adult male patients suffering from acute, uncomplicated falciparum malaria admitted to the Bangkok Hospital for Tropical Diseases, Mahidol University, Bangkok, Thailand from March to November 1989. The hospital is situated in a non-malarious area. Informed consent was obtained from the patients before they were entered into the trial. Exclusion criteria were females, age below 14 years, mixed

infection, high parasitemia (over 100,000/ μ l), concurrent diseases, history of recent antimalarial treatment or positive urine tests for sulfonamides and/or 4-aminoquinolines. The study was approved by the Ethical Committee, Faculty of Tropical Medicine, Mahidol University. A total of 68 patients participated in the trial.

Each patient underwent a complete physical examination on admission. Baseline hematology and biochemistry tests including glucose-6-phosphate dehydrogenase and hemoglobin type determinations were performed. Parasite counts of both asexual forms and gametocytes of *P. falciparum* were determined prior to treatment, every 6 hours during parasitemia and then daily throughout the observation period of 28 days. Signs, symptoms and vital signs were recorded every 4 hours. Electrocardiograms were recorded prior to drug administration and on days 2, 4, 7, 14, 21 and 28. White cell count and reticulocyte count were performed daily for 28 days.

In vitro sensitivity of *P. falciparum* to chloroquine, quinine, quinidine and mefloquine was determined by the microtechnique (Rieckmann *et al*, 1978).

Study design and evaluation

The patients were assigned randomly to groups A, B, C, D, A1, B1, AB, AC, or ASP, the groups being defined thus:

- Group A Artesunate 1,200 mg orally in 5 days (initial 2 doses of 200mg followed by 8 doses of 100mg every 12 hours).
- Group B Artesunate 600mg orally in 5 days (half dose of group A).
- Group C Artemether 480mg intramuscularly in 5 days (initial dose 160mg followed by 4 doses of 80mg daily).
- Group D Artesunate 300mg intravenously in 3 days (initial dose 120mg followed by 60mg 4 hours later, then once daily for the next 2 days).
- Group A1 Artesunate 600mg orally in 2 days (100mg every 8 hours for 6 doses).
- Group B1 Artesunate 600mg orally in 1 days, (200mg every 12 hours for 3 doses).
- Group AB Artesunate 650mg orally in 5 days (initial dose 200mg followed by 9 doses of 50mg every 12 hours).

Group AC Artesunate 200mg orally as a single dose, 6 hours later a standard dose chloroquine (1,500mg base in 48 hour-treatment course).

Group ASP Artesunate 200mg orally as a single dose, 6 hours later 3 tablets of Fansidar[®] (1,500mg sulfadoxine and 75mg pyrimethamine).

Artesunate tablets (50mg/tablet) and intravenous artesunate (60mg/vial) were supplied by Prof GQ Li of the College of Traditional Chinese Medicine, Guangzhou, China. Artesunate for intravenous injection was dissolved in 1ml 5% NaHCO₃ + 5 ml normal saline injected over 3 minutes. If *P. vivax* infection became patent during follow-up a single dose of 150 mg base of chloroquine was given for temporary relief, followed by a full therapeutic course with primaquine after day 28.

Parasite clearance time and fever clearance time in each treatment group were calculated as described previously (Harinasuta *et al*, 1983, 1987) and compared by Students *t*-test. As platelet and parasite counts were not normally distributed; they were compared by the Kruskal-Wallis, one way ANOVA test. The parasite reductions at different times after treatment were evaluated by one-way ANOVA test (repeated measurements).

RESULTS

The clinical and laboratory findings of the nine groups of patients admission are shown in Tables 1 and 2.

Each group consisted of 5 to 6 patients initially. Although good responses were obtained in groups A, B, C and AB, an additional 5 and 15 patients were recruited only to group B and AB. A total of 68 patients took part in the trial but only 65 patients completed the 28-day follow-up period. After they had recovered, three patients (1 each from groups A, C and AB) dropped out on day 14, 9, and 3 for personal reasons not related to drug administration.

Clinical response

All 68 patients had excellent initial clinical response. Symptoms disappeared after 1 to 3 days of treatment. Fever and parasite clearance times

Table 1
Clinical parameters.

Group	A	B	C	D	A1	B1	AB	AC	ASP
No. of patients	6	10	6	5	5	5	21	5	5
Age (y) +	27.2 ± 6.8 (19-39)	26.8 ± 8.5 (18-44)	22.7 ± 7.1 (15-35)	22.0 ± 3.3 (18-25)	23.4 ± 7.5 (25-35)	23.0 ± 5.4 (18-31)	23.8 ± 6.4 (16-43)	23.8 ± 4.7 (17-27)	21.0 ± 4.5 (18-29)
Weight (kg)	53.5 ± 5.5 (47-59)	53.7 ± 4.9 (45-62)	49.6 ± 3.6 (45-55)	50.7 ± 3.7 (45-55)	54.7 ± 4.3 (48-58)	52.0 ± 3.8 (48-56)	52.1 ± 4.2 (42-60)	53.9 ± 7.1 (47-63)	56.0 ± 4.0 (51-60)
Height (cm)	164.0 ± 6.8 (154-174)	164.0 ± 4.9 (156-174)	161.3 ± 5.6 (153-169)	165.2 ± 3.4 (162-171)	164.4 ± 8.3 (151-172)	160.4 ± 5.3 (155-168)	164.1 ± 5.8 (152-172)	161.7 ± 9.0 (154-173)	167.2 ± 8.6 (155-178)
Duration of Fever (days) before admission	8 ± 3.0 (1-10)	2.9 ± 2.0 (1-7)	3.1 ± 0.9 (2-5)	2.0 ± 0.7 (1-3)	3.2 ± 1.4 (1-5)	4.0 ± 3.6 (1-10)	5.9 ± 4.8 (1-20)	4.6 ± 3.2 (2-10)	4.4 ± 2.4 (2-7)
Temperature °C Before treatment	38.6 ± 0.5 (37.8-39.2)	38.7 ± 0.8 (37.9-40.5)	39.1 ± 0.3 (38.7-39.5)	39.7 ± 0.5 (39.2-40.5)	38.8 ± 1.0 (37.5-39.9)	38.2 ± 0.3 (37.8-38.5)	39.1 ± 0.6 (37.5-40.5)	39.0 ± 0.4 (38.5-39.5)	39.2 ± 0.9 (38.7-40.2)

Data expressed as mean ± SD; (range)

Table 2
Laboratory findings on admission.

Group	A	B	C	D	A1	B1	AB	AC	ASP
No. of patients	6	10	6	5	5	5	21	5	5
Hemoglobin (gm%)	11.9 ± 2.6 (9.1-15.3)	12.9 ± 2.1 (9.7-15.2)	11.4 ± 1.7 (8.4-13.7)	12.1 ± 1.7 (9.8-13.7)	11.9 ± 2.3 (5.6-14.1)	12.7 ± 1.3 (11.1-14.1)	11.6 ± 2.9 (6.2-17.9)	12.8 ± 3.9 (7.1-16.7)	11.0 ± 2.6 (7.0-14.3)
Hematocrit (%)	36.6 ± 7.7 (24-46)	38.8 ± 6.0 (31-45)	36.0 ± 5.8 (27-44)	37.2 ± 6.0 (29-43)	35.6 ± 6.8 (25-40)	38.6 ± 2.8 (35-42)	35.2 ± 8.5 (20-52)	36.8 ± 11.2 (20-48)	34.4 ± 8.9 (21-46)
White blood cell Count × 10 ³ /μl	7.4 ± 3.1 (2.4-11.0)	7.1 ± 2.1 (4.5-11.3)	5.8 ± 3.2 (3.2-10.3)	4.8 ± 1.1 (3.9-6.6)	5.5 ± 1.8 (3.7-8.1)	6.9 ± 2.6 (3.2-10.3)	5.8 ± 1.9 (2.6-8.9)	6.2 ± 2.6 (2.7-8.9)	4.8 ± 1.5 (3.2-7.0)
Geometric mean Parasite count × 10 ⁴ /μl	4376	3950	4334	9794	6301	4173	4340	4391	4173
Blood urea	13.4 ± 4.1 (5.5-16.7)	14.0 ± 3.6 (10.0-21.2)	13.2 ± 3.5 (8.0-19.2)	15.9 ± 6.0 (11.0-26.0)	11.7 ± 4.5 (8.6-20.0)	14.3 ± 4.5 (7.3-24.0)	14.1 ± 4.7 (12.5-17.5)	15.3 ± 2.4 (11.3-33.6)	15.3 ± 4.5 (11.3-22.6)
Nitrogen (mg/dl)	1.0 ± 0.2 (0.8-1.5)	1.2 ± 0.2 (0.9-1.5)	1.2 ± 0.2 (1.0-1.5)	1.2 ± 0.2 (0.7-1.3)	1.2 ± 0.2 (0.9-1.5)	1.2 ± 0.2 (0.9-1.4)	1.2 ± 0.1 (0.9-1.6)	1.2 ± 0.1 (1.2-1.4)	1.1 ± 0.2 (0.9-1.5)
Total bilirubin (mg/dl)	1.6 ± 1.8 (0.4-5.2)	1.3 ± 0.6 (0.8-2.3)	1.5 ± 0.7 (0.4-2.4)	1.8 ± 0.6 (0.9-2.6)	0.8 ± 0.2 (0.4-1.1)	1.4 ± 0.6 (0.6-2.0)	1.2 ± 0.7 (0.3-2.5)	1.3 ± 0.6 (0.6-2.2)	2.9 ± 1.2 (1.3-4.1)
Serum aspartate Aminotransferase*	29 ± 5.6 (22-35)	29 ± 7.3 (18-42)	26 ± 4.1 (20-31)	23 ± 2.1 (20-25)	31 ± 6.7 (24-39)	26 ± 5.2 (21-35)	41 ± 37.0 (20-198)	31 ± 3.8 (27-35)	29 ± 7.0 (18-35)
Serum alanine Aminotransferase*	23 ± 4.0 (11-26)	26 ± 8.2 (14-37)	20 ± 3.0 (15-24)	18 ± 1.9 (16-21)	28 ± 9.8 (18-42)	22 ± 5.2 (20-32)	37 ± 46 (16-232)	26 ± 4.5 (21-32)	21 ± 3.5 (16-26)
Albumin (g/dl)	3.6 ± 0.6 (3.3-4.2)	4.2 ± 0.2 (3.9-4.5)	3.9 ± 4.0 (3.1-4.4)	3.8 ± 0.3 (3.2-4.3)	4.4 ± 1.3 (3.2-6.7)	3.9 ± 0.4 (3.6-4.5)	3.5 ± 0.4 (2.8-4.4)	3.8 ± 0.7 (3.0-4.5)	3.6 ± 0.3 (3.2-4.0)
Globulin (g/dl)	2.8 ± 0.3 (2.3-3.2)	2.8 ± 0.3 (2.5-3.6)	3.3 ± 0.4 (2.5-2.9)	2.8 ± 0.2 (2.4-3.0)	2.9 ± 0.2 (2.6-3.3)	2.4 ± 0.1 (2.2-2.7)	2.9 ± 0.4 (2.2-3.7)	3.1 ± 0.3 (2.8-3.6)	2.9 ± 0.3 (2.5-3.3)

Data expressed as Mean ± SD; (range)

* Reitman - Frankel units/ml

ARTESUNATE AND ARTEMETHER IN RESISTANT MALARIA

Table 3
Therapeutic response.

Group	A	B	C	D	A1	B1	AB	AC	ASP
Total dose.....mg	1200 PO	600 PO	480 IM	300 IV	600 PO	600 PO	650 PO	200 PO+CQ	200 PO+SP
Duration.....days	5	5	5	3	2	1	5	1+2	1
No. of patients	6	10	6	5	5	5	21	5	5
Fever clearance time (hour)	42.0 ± 15.6 (28-64)	24.4 ± 18.1 (0-64)	32.5 ± 19.7 (10-55)	25.6 ± 10.6 (16-41)	26.8 ± 17.5 (19-56)	39.2 ± 15.5 (24 ² 62)	24.0 ± 8.2 (12-36)	21.4 ± 11.0 (7-34)	23.4 ± 6.7 (16-30)
Parasite clearance time (hour)	31.0 ± 7 (24-42)	27.0 ± 7.6 (18-42)	29.0 ± 7.0 (24-42)	33.6 ± 6.8 (24-42)	27.6 ± 9.0 (18-42)	28.8 ± 6.5 (24-36)	27.6 ± 8.3 (18-48)	31.2 ± 5.0 (24-36)	31.2 ± 14.9 (18-54)
No. of pt completed 28 dFU	5	10	5	5	5	5	20	5	5
No. of pt with S response (%)	5 (100)	9 (90)	5 (100)	1 (20)	0 (0)	0 (0)	19 (95)	0 (0)	0 (0)
No. of pt with RI response (%)	0 -	1 (10)	0	4 (80)	5 (100)	5 (100)	1 (5)	5 (100)	5 (100)
Recrudescence on days		20		13,13,14,19	12-20	10-24	20	7-12	7-21
No. of pt had vivax Parasitaemia (on day)	1 (17)	2 (24, 28)	1 (22)	0 (0)	-	-	8 (12-28)	-	-

PO = per oral
IM = intramuscular
IV = intravenous
CQ = chloroquine 1500mg base
SP = sulfadoxine 1500mg + pyrimethamine 75 mg
Data = expressed as Mean ± SD; (range)

were short (Table 3) and the time taken to clear 99% parasitemia was 18 to 36 hours.

The parasitemia declined in a sigmoidal curve after treatment in all except three patients. However, even in these patients parasitemia was eventually reduced without any additional intervention after 12 hours of treatment and all of them were cured. The mean level of parasitemia at 6, 12, 18 hours showed a consistent reduction pattern after treatment with significant differences over each interval ($p < 0.05$).

The cure rates in groups A and C were 100%, group B 90%, group AB 95% and group D 20%. No cure was obtained with the other treatment regimens. All treatment failures were of the RI response no RII or RIII (WHO, 1973) responses were observed.

During the 28-day follow-up of 39 patients who responded to *P. vivax* infection became patent in 12 (31%). The infection was successfully suppressed with 150mg base of chloroquine.

Adverse effects

The drugs were well tolerated up to a total dose of 1200 mg which was double the recommended dose. No patients in the oral treatment groups had any gastrointestinal upset. A few patients in the IM group (group C) complained of pain at the injection site.

There were no cardiovascular side-effects except for minor abnormalities in the electrocardiograms in some patients. These returned to normal within seven days. Blood pressure and pulse rate showed no drug-associated changes.

Erythrocyte counts, hematocrit levels, and reticulocyte counts did not show any drug-associated changes. But reduction of neutrophils by more than 20% was noted on days 2, 4 in groups A, C and D (1200 mg oral artesunate IM artemether and IV artesunate). These were transient and all of the patients were well and had rapid convalescence.

In vitro tests

Fifty-two isolates were successfully cultured and tested for chloroquine, quinine, quinidine and mefloquine (Table 4). All the isolates were resistant to chloroquine according to WHO criteria (WHO,1984). The mean minimum inhibitory concentration (MIC) values for quinine 3.2 umol/l and quinidine 1.6 umol/l were higher than reported in 1987 (Bunnag *et al*, 1987).

DISCUSSION

Several oral dosages of artesunate were tested, group B as well as AB gave promising results therefore another 5 and 15 patients were included

Table 4

In vitro minimum inhibitory concentrations (pmol/50 µl).

	Mefloquine (n = 52)	Chloroquine (n = 51)	Quinine (n = 50)	Quinidine (n = 51)
Mean	15.0	54.9	32	78.1
± SD	11.4	47.8	165.0	70.1
Range	(4-64)	(16-256)	(16-1024)	(8-256)

in B and AB groups respectively. Further studies using artemether will be carried out at a later date.

All patients in this study had fever clearance times and parasite clearance times (Table 3) shorter than those experienced in patients who were treated either by mefloquine or quinine in Thailand; 29.26 ± 21.29 and 49.67 ± 21.14 hours in mefloquine 1,000 mg (Harinasuta *et al*, 1983), 43 ± 6 and 83 ± 5 hours in quinine sulfate 600mg tid for 5 days (Suntharasamai *et al*, 1984) respectively. These clearance times were comparable to those reported from studies in China (Jiang *et al*, 1982; Li *et al*, 1984).

The cure rates obtained from this study were 90-100% in groups A, B, C, AB comparing with only 0-20% in groups D, A1, B1. The discrepancy of these cure rates may be due to the pharmacokinetic properties of the drug formulations, the half-life being 10-12 hours for the oral and intramuscular routes whereas it is only 30-45 minutes for the intravenous route (Li GQ, personal communication). Moreover, the duration of the drug treatment has obviously played a significant role as all successful regimens exceeded three days. It is highly suggestive that at least 3 cycles of blood schizogony are required to be totally eradicated the asexual forms. Chloroquine (later known to be antagonistic to artesunate, Chawira and Warhurst, 1987) and Fansidar given after a single-dose of oral artesunate treatment had no additional effect on cure rate in patients who showed RI type of responses which required treatment with quinine and tetracycline for seven days.

Twelve out of thirty-nine (31%) patients who were successfully treated with oral artesunate and intramuscular artemether (groups A, B, C, AB) had vivax malaria during 28 days follow up. The rate of relapses of *P. vivax* infection may be due to the short half-life of the compounds. These

data also suggested that the drug had no action against hypozoites. This result emphasized the importance of the follow-up after initial successful treatment (Looareesuwan *et al*, 1987).

Oral and intravenous artesunate and intramuscular artemether are well tolerated and the incidence of side effects was very low. Further studies should be carried out to determine optimum dosage and duration of treatment for multidrug resistant falciparum malaria. Also potential hematological consequences of treatment with artemisinin drugs merit further studies and follow-up at longer term.

ACKNOWLEDGEMENTS

We are grateful to Professor GQ Li, College of Traditional Medicine, Guangzhou, China and Dr Keith Arnold, Roche Asian Research Foundation, Hong Kong for the donation of artesunate and artemether, and to Atlantic Co Ltd, Thailand, and Guilin Pharmaceutical Works China for oral artesunate and part of the financial support for the study. We also are grateful to Prof WH Wernsdorfer and Dr May Ho for reading this manuscript.

REFERENCES

- Bunnag D, Harinasuta T. The current status of drug resistance in malaria In : Howell MJ, ed. Parasitology Quo Vadit? Proceedings of the 6th International Congress of Parasitology. Australian Academy of Science, 1986 : 169-80.
- Bunnag D, Harinasuta T, Vanijanonta S, Looareesuwan S, Chittamas S, Punnavut W, Jochims E. Slow-release quinidine in the treatment of chloroquine resistant falciparum malaria : a double-blind trial. *Acta Leidensia* 1987; 55 : 129.

ARTESUNATE AND ARTEMETHER IN RESISTANT MALARIA

- Chawira AN, Warhurst DC. The effect of artemisinin combined with standard antimalarials against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* in vitro. *J Trop Med Hyg* 1987; 90 : 1-8.
- Harinasuta T, Suntharasamai P, Viravan C. Chloroquine-resistant falciparum malaria in Thailand. *Lancet* 1965; 2 : 657-60.
- Harinasuta T, Bunnag D, Wernsdorfer WH. A phase II clinical trial of mefloquine in patients with chloroquine-resistant falciparum malaria. *Bull WHO* 1983; 62 : 299-305.
- Harinasuta T, Bunnag D, Vanijanond 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; 65 : 363-7.
- Jiang JB, Li GQ, Guo XB, Kong YC, Arnold K. Antimalarial activity of mefloquine and qinghaosu. *Lancet* 1982; 2 : 285-8.
- Klayman DL. Qinghaosu (artemisinin) : An antimalarial drug from China. *Science* 1985; 228 : 1049-55.
- Li GQ, Arnold K, Guo XB, Jian HX, Fu LC. Randomised comparative study of mefloquine, qinghaosu and pyrimethamine-sulfadoxine in patients with falciparum malaria. *Lancet* 1984; 2 : 1360-1.
- Looareesuwan S, White NJ, Chittamas S, Bunnag D, Harinasuta T. High rate of *Plasmodium vivax* relapse following treatment of falciparum malaria in Thailand. *Lancet* 1987; 2 : 1052-4.
- Myint PT, Shew T. The efficacy of artemether (Qinghaosu) in *Plasmodium falciparum* and *P. vivax* in Burma. *Southeast Asian J Trop Med Public Health* 1987; 17 : 223-5.
- Qinghaosu Antimalarial Coordinating Research Group. Antimalaria studies on Qinghaosu. *Chin Med J* 1979; 92 : 811-4.
- Rieckmann KH, Sax LJ, Campbell GM, Mrema JE. Drug sensitivity of *Plasmodium falciparum*. An in vitro microtechnique. *Lancet* 1978; 1 : 22-3.
- Reacher M, Campbell CC, Freeman J, Doberstyn EB, Brandling-Bannett AD. Drug therapy of *Plasmodium falciparum* malaria resistant to pyrimethamine sulfadoxine (Fansidar). *Lancet* 1981; 2 : 1066-8.
- Shwe T, Myint PT, Htut Y, Myint W, Soe L. The effect of mefloquine-artemether compared with quinine on patients with complicated falciparum malaria. *Trans R Soc Trop Med Hyg* 1988; 82 : 665-7.
- Suntharasamai P, Vanijanond S, Harinasuta T, Bunnag D. A double-blind randomised trial of quinine VS quinidine in chloroquine resistant falciparum malaria. Symposium on quinidine on falciparum malaria. Bangkok, June 1984. Abst : 15.
- WHO Scientific Group. Advance in malaria chemotherapy. *WHO Tech Rep Ser* 1984; 711.