ARTEMETHER-PYRIMETHAMINE IN THE TREATMENT OF PYRIMETHAMINE-RESISTANT FALCIPARUM MALARIA

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Abstract. *In vitro* susceptibility and clinical response of multidrug resistant *Plasmodium falciparum* to the combination artemether-pyrimethamine were evaluated in patients with acute uncomplicated falciparum malaria. Sixty patients were randomized to receive 3 oral regimens of the combination artemether-pyrimethamine as follows: *Regimen-I*: artemether (300mg) plus pyrimethamine (100mg) on the first day, then placebo on the two consecutive days; *Regimen-II*: artemether (300mg) plus pyrimethamine (100mg) on the first day, then artemether (150mg) plus pyrimethamine (50mg) on the second day, and placebo on the third day; *Regimen-III*: artemether (300mg) plus pyrimethamine (100mg) on the first day, then artemether (150mg) plus pyrimethamine (50mg) on the second and third days. All patients had a rapid initial response to treatments with 95% of parasitemia being cleared within the first 24 hours. $PCT_{24hours}$ and $PCT_{48hours}$ were similar among the three drug regimens (11 *vs* 4, 6 *vs* 12, and 9 *vs* 11 patients for a 1-day, 2-day, and 3-day combination regimen, respectively). Fever was cleared within 48 hours in all patients in either group. Transient mild nausea, vomiting and loss of appetite were found in a few patients during the first 2 days of treatment. Seven patients did not complete the 28 day follow-up period (5 *vs* 2 in a 1-day *vs* 2-day regimen), the reason for withdrawal was not associated with drug-related adverse effects. Only 53 patients were therefore qualified for the efficacy assessment. There was 15, 13 and 5 patients in a 1-day, 2-day and 3-day combination regimens, respectively, who had reappearance of the parasitemia between days 11 and 21. The cure rates of the 3 treatment groups were statistically significantly different (0, 27.8, and 75% for a 1-day, 2-day and 3-day combination regimen, respectively). Two patients developed *P. vivax* malaria on days 20 and 24. All of the isolates were highly resistant to pyrimethamine, with MIC of 10^{-5} M. There is potential advantage of this combination therapy in reducing the dosage and treatment period of artemisinin derivative, which is therefore likely to improve complaince in clinical practice. The use of a 3-day combination regimen (300mg artemether plus 100mg pyrimethamine on the first day, then 150mg artemether plus 50mg pyrimethamine on the second and third days) seems to be a good alternative regimen to sulfadoxine/pyrimethamine in areas where *P. falciparum* is sensitive to pyrimethamine *eg* in Africa.

INTRODUCTION

Malaria remains a major health problem of the world, particularly in the tropics (WHO, 1993). Failure to control the disease is due mainly to the emergence of drug resistance of *Plasmodium falciparum* (Karbwang and Harinasuta, 1992a; Wernsdorfer, 1994). In Southeast Asia, resistance of the parasite to chloroquine, sulfadoxine-pyrimethamine, quinine and mefloquine are well documented both *in vitro* and *in vivo* (Harinasuta *et al*, 1965; Karbwang *et al*, 1994a; Ketrangsee *et al*, 1992; Pinichpongse *et al*, 1982). With this deteriorating situation, one of the strategies aimed at controlling the disease is through modification of antimalarial drug regimens or the use of drug combinations. The rationale of using combination therapy is to exploit pharmacologically additive or synergistic effects with acceptable patient compliance. In the past, several antimalarial combinations have been used and shown to improve the therapy of clinical falciparum malaria. These include the combinations of sulfadoxine/pyrimethamine (Fansidar®), quinine/tetracycline, mefloquine/sulfadoxine/pyrimethamine (MSP; Fansimef®), mefloquine/tetracycline, artemether/mefloquine *etc* (Bunnag *et al*, 1991a; 1995; Harinasuta *et al*, 1987; Karbwang *et al*, 1994b; 1995). The combination of artemisinin compounds with pyrimethamine, which act on different stages of parasite development and resides in the body over a longer period of time, might be expected to improve radical cure rate, with a shorter duration of treatment. Although *P.*

falciparum strains in Thailand are insensitive to pyrimethamine (Karbwang and Harinasuta, 1992a), using this drug in combination with artemisinin compounds may improve its efficacy as that seen with the combination of artemether or artesunate with mefloquine (Bunnag *et al*, 1995; Karbwang *et al*, 1995).

In the present study, *in vitro* susceptibility and clinical response of multidrug resistant *P. falciparum* to the three combination regimens of artemether-pyrimethamine were evaluated in patients with acute uncomplicated falciparum malaria, with an attempt to define the most effective and shortest course regimen of this combination.

MATERIALS AND METHODS

Patients and methods

The study was carried out in 1995 during the rainy season (June-August). Patients presenting to the out-patient malaria clinic in Mae Sot, Tak Province (along the Thai-Myanmar border) were screened for *Plasmodium falciparum* infections. This part of the country is well-documented as a multidrug resistant area. Uncomplicated malaria patients aging between 15 and 55 years were recruited to the study provided that informed consent for participation was obtained. On admission, all patients under went physical examination, monitoring of baseline general symptoms and body temperature. The criteria for inclusion were as follows; the presence of asexual form parasitemia lower than 100,000 per μl of blood, and the absence of history of mefloquine or quinine treatment over the last 4 weeks confirmed by drug levels determined in blood using high performance liquid chromatography (Karbwang *et al*, 1991a; b). The patients were randomized to receive three oral drug regimens as follows:

Regimen I: a 1-day regimen of artemether-pyrimethamine, consisting of artemether (300mg : Artenam®; Arenco nv, Belgium) plus pyrimethamine (100mg : Daraprim®; Wellcome) on the first day, then placebo on the 2 consecutive days;

Regimen II: a 2-day regimen of artemether-primethamine, consisting of artemether (300mg) plus pyrimethamine (100mg) on the first day, then artemether (150mg) plus pyrimethamine (50mg) on the second day, and placebo on the third day.

Regimen III: a 3-day regimen of artemether-pyrimethamine, consisting of artemether (300mg) plus pyrimethamine (100mg) on the first day, then artemether (150mg) plus pyrimethamine (50mg) on the second and third days.

The patients were out of malaria endemic areas for 28 days (duration of study). During the first 3 days, they were admitted as in-patients at the malaria clinic.

Parasite counts, body temperature and adverse effect monitoring were performed daily until 3 days, then once weekly for 4 weeks (*ie* days 7, 14, 21 and 28). Parasite counts (per 1,000 red blood cells or per 200 white blood cells) were done using thin and thick blood films stained with Giemsa's stain. *In vitro* sensitivity test for pyrimethamine was performed in all patients prior to the treatment, using the standard *in vitro* technique of Rieckman *et al* (1979) with modifications.

Only those patients who remained out of malaria endemic areas for 28 days and completed the 28-day follow-up period were included for efficacy assessment. Clearance of peripheral blood parasitemia during the first and second days of treatment ($PCT_{24hours}$ *vs* $PCT_{48hours}$) was recorded in all patients. The cure rate was used as primary parameter in the evaluation of the clinical efficacy of each regimen. Patients with negative blood slides until day-28 were considered to have an S (sensitive) type of response, whereas those with negative blood slides until day-7 but had reappearance afterward were considered to have RI type of response (resistance grade-I). Cases showing of a reduction of asexual parasitemia of more than 50% on day-2 but persistence until day-7, were classified as RII type of response (resistance grade-II), while no marked reduction of asexual parasitemia (< 50%) on day-2 was classified as RIII type of response (resistance grade-III). Comparison of clinical cure rate between the two treatment regimens was done by Chi-square test, with a statistically significant level of $p = 0.05$.

Patients were asked to return to the clinic if they had reappearance of fever. In case of treatment failure, patients were retreated with the combination regimen of artemether-mefloquine (300mg artemether plus 750 and 500 mg mefloquine, given at 24 and 30 hours after artemether). Those who developed *P. vivax* malaria during the follow-up

period were given 150mg (base) of chloroquine to suppress the symptoms and received a full course of treatment on discharge.

RESULTS

Sixty patients (45 males, 15 females) with acute uncomplicated falciparum malaria were recruited into the study, 20 patients in each regimen. All patients presented with fever (body temperature above 37.3°C), headache and/or anorexia or vomiting on admission. None had detectable baseline blood level of mefloquine or quinine. The levels of admission parasitemia were not statistically significantly different between the 3 treatment groups (Table 1).

Seven patients did not complete the 28 day follow-up period (5 *vs* 2 in a 1-day *vs* 2-day combination regimen); the reason for withdrawal was not associated with drug-related adverse effects. Only 53 patients were therefore qualified for the efficacy assessment.

All patients had a rapid initial response to treatments with 95% of parasitemia being cleared within the first 24 hours. The number of patients with $PCT_{24hours}$ and $PCT_{48hours}$ were similar among the three drug regimens (11 *vs* 4, 6 *vs* 12, and 9 *vs* 11 patients for a 1-day, 2-day, and 3-day combination regimen, respectively). There were 15, 13 and 5 patients in a 1-day, 2-day and 3-day combination regimens, respectively, who had reappearance of the parasitemia between days 11 and 21. The cure rates for a 1-day, 2-day and 3-day combination regimens were 0% (95% CI = 0-0%), 27.8% (95% CI = 9.7-53%), and 75% (95% CI = 50.8-91.3%), respectively (Table 1). The cure rates of the 3 treatment groups were statistically significantly different (1-day *vs* 2-day regimen: p = 0.005, RR 1.38, 95% CI 1.04-1.84; 1-day *vs* 3-day regimen: p = 0.0004, RR 4.0, 95% CI 1.05-2.2; 2-day *vs* 3-day regimen: p = 0.01, RR 2.89, 95% CI 1.28-6.5). Fever was cleared within 48 hours in the patients in all groups. Transient mild nausea, vomiting and loss of appetite were observed in a few patients during the first 2 days of treatment. There were 2 patients who developed *P. vivax* mala-ria on days 20 and 24.

The *in vitro* sensitivity test was successful in 31 isolates. All of the isolates showed resistance to pyrimethamine; no inhibition was seen even at the concentration of as high as 10^{-5}M (minimum inhibitory concentration; MIC).

DISCUSSION

Pyrimethamine is an antifolate antimalarial which is active against several stages of human malaria *ie* tissue and erythrocytic stages. Its action on the late phase (trophozoites and schizonts) ery-

Table 1

Clinical data on admission and response to treatment with the 3 regimens of artemether-pyrimethamine combination (data were presented as median (range) values or number).

	1-day combination	2-day combination	3-day combination
Age (years)	22 (15-34)	20.5 (15-50)	20 (15-40)
RBC ($\times 10^{12}$ per µl)	4.35 (2.13-5.36)	4.65 (2.56-7.14)	4.39 (2.57-6.4)
Admission parasitemia (per µl)	10,650 (1,000-80,250)	48,460 (3,460-562,650)	21,450 (960-105,000)
Number of patients included	15	18	20
S response (n)	0	5	15
R-I response (n)	15 (days 11-21)	13 (days 7-17)	5 (days 14-21)
Cure rate (%)	0	27.8	75
$PCT_{24hours}$(n)	11	6	9
$PCT_{48hours}$(n)	4	12	11
P. vivax (n)	0	0	2 (days 20, 24)

throcytic stage is slow and thus, the use of pyrimethamine alone is limited in acute falciparum malaria. The combination of pyrimethamine with sulfonamides *eg* sulfadoxine (SP: Fansidar®) has been used as a major chemotherapeutic agent in chloroquine-resistant areas in Africa. In Southeast Asia and South America however, resistance to this combination has emerged and spread (Wernsdorfer and Payne, 1991). The increasing evidence of highly multidrug resistant *P. falciparum* in these areas has led to the use of artemisinin and derivatives, a new class of antimalarial drugs that have been shown to be highly effective, even against strains that have developed resistance to several currently available drugs (Bunnag *et al*, 1991b; 1992; Karbwang *et al*, 1992b). Artemether is one of artemisinin derivatives which is a potent antimalarial with rapid onset of action against an early stage (tiny rings) of asexual parasites (Li *et al*, 1982). Nevertheless, in order to achieve more than 90% cure rate, the duration of treatment needs to be at least 5 days with a total dose of at least 600mg. These prolonged treatment courses are likely to reduce patient compliance, or increase the cost of therapy. Combination of the rapid therapeutic effect of artemether with drug that acts upon different stages of erythrocytic phase of the parasite like pyrimethamine has been considered more logical approach than the monotherapy.

In the present study, we have avaluated the clinical efficacy of the combination of artemether-pyrimethamine in multidrug resistant *P. falciparum*. Although a previous *in vivo* study in mice has demonstrated that artemisinin compounds antagonize the effect of sulfadoxine and pyrimethamine (Chawira *et al*, 1987), it was clearly shown in our preliminary study that artemether exhibited additive, rather than antagonistic interaction with pyrimethamine (Tan-ariya, unpublished observation). This is in agreement with the current observation showing the improved clinical efficacy of the combination comparing to each individual drug alone, despite the markedly low *in vitro* susceptibility of the parasites *in vitro* (MIC = 10^{-5} M). In addition, the coadministration of artemether with pyrimethamine resulted in shorter parasite clearance time than that reported in previous studies with sulfadoxine/pyrimethamine (Doberstyn *et al*, 1976). In most cases, 95% of peripheral parasitemia was cleared within the first 24 hours of treatment.

In Thailand, *P. falciparum* strains are highly resistant to both sulfadoxine and pyrimethamine; the efficacy of the combined drug is virtually 0% (Karbwang and Harinasuta, 1992a). Furthermore, monotherapy with artemether over 1-3 days was associated with low radical cure rate of 0% (Bunnag *et al*, 1991b). Bunnag *et al* (1991b) also reported that a 1-day combination regimen of artesunate (another artemisinin derivative), with sulfadoxine/pyrimethamine (300, 1,500 and 75mg) was totally ineffective (100% recrudescence rate). When artemether and pyrimethamine were given together in this study, radical cure rate was improved with a 2 or 3-day, but not with a 1-day combination regimen (Cure rates of 27.8, 75 and 0%, respectively). The superiority of the combination over each individual drug alone could be due to sum effects of both drugs, as they have specific actions on different stages of the parasites. In addition, since pyrimethamine resides in the body at a longer time than does artemether (half-life of 2-5 days *vs* 2-6 hours, Na-Bangchang *et al*, 1994; Weidekamm *et al*, 1982), the sustained therapeutic drug concentrations for falciparum malaria would be expected.

In conclusion, a combination of artemether with pyrimethamine at 3-day duration is more effective than artemether alone even in the area of pyrimethamine-resistance, indicating by a reduction of recrudescence rate while minimally affecting the tolerability profile. There is a potential advantage of this combination therapy in reducing the dosage and treatment period of artemisinin derivatives, which is therefore likely to improve compliance in clinical practice. Although the cure rate of a 3-day combination regimen (300mg artemether plus 100mg pyrimethamine on the first day, then 150mg artemether plus 50mg pyrimethamine on the second and third days) is not good enough to be used in areas with high degree of pyrimethamine-resistance, it seems to be a good alternative regimen to sulfadoxine/pyrimethamine in areas where *P. falciparum* is sensitive to the drug *eg* in Africa. Investigation of clinical efficacy of this combination in areas with pyrimethamine-sensitive *P. falciparum* strains is encouraging.

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