PHASE II TRIAL IN CHINA OF A NEW, RAPIDLY-ACTING AND EFFECTIVE ORAL ANTIMALARIAL, CGP 56697, FOR THE TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA

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Abstract. One hundred and two Chinese out-patients with naturally acquired, previously untreated, falciparum malaria were selected to evaluate the efficacy of a new combination anti-malarial therapy, CGP 56697 (artemether plus benflumetol). In this open non-comparative trial each patient received a combination of 80mg artemether and 480mg benflumetol given orally at 0, 8, 24 and 48 hours (total: 320mg artemether, 1,920mg benflumetol). Patients were kept for 28 days in a transmission-free hospital in an area with chloroquine resistant falciparum malaria to prevent reinfection and to aid diagnosis of recrudescence. Progress and possible adverse effects were monitored by blood film parasitology, blood biochemistry assays, urinalysis, ECG and X-ray. Ninety-eight of the 102 patients were shown to be free of infection at 28 days, a 96.1% cure rate. Parasite reduction at 24 hours was 99.4%. Time to effect complete parasite clearance ranged from 24 to 54 hours (median 30 hours). Time for fever clearance ranged from 6 to 78 hours (median 18 hours). Recrudescence was low (3.9%). No significant adverse side-effects were encountered. It is concluded that CGP 56697, a combination anti-malarial therapy of artemether with benflumetol, offered a rapid and highly effective treatment for acute uncomplicated falciparum malaria in an area of chloroquine-resistant malaria in China.

INTRODUCTION

With the current estimates of 300 million to 500 million new malaria cases arising worldwide, each year, and the attendant 1.5 to 2.7 millon annual deaths (WHO, 1995), the rapid escalation in drug resistant forms of malarial parasite species poses enormous health problems.

From the 17th century onwards quinine has been the mainstay treatment for malaria. Following World War II, in the late 1940s, the synthetic anti-malarial chloroquine replaced quinine as the first choice treatment and prophylactic antimalarial, but within little more than a decade chloroquine-resistant forms were identified in Thailand and Cambodia. These are now found extending to other endemic regions. Chloroquine-resistant falciparum malaria is now a major problem in Asia and resistant forms in Africa and Latin America are rapidly emerging. A similar picture has emerged with the more recent chloroquine successors, such as mefloquine.

There is a reported 100% resistance of *P. falciparum* to chloroquine and primaquine in Thailand, and partial resistance to all other anti-malarials, for example up to 40% resistance to mefloquine (Looareesuwan *et al*, 1992).

Mefloquine is still the drug of choice in most regions where chloroquine resistant falciparum malaria has evolved, but there is growing evidence of the seriousness of the adverse neuropsychiatric side-effects associated with the use of this drug in a small proportion of patients. Furthermore, mefloquine resistant *P. falciparum* malaria is now increasingly reported to be spreading all over South-east Asia (WHO, 1995).

In particular it is the increasing drug resistance and widening geographic distribution of this most lethal of the malarial parasites, *Plasmodium falciparum*, that has directed attention towards the urgent need for development of novel anti-malarial drugs.

A recent approach has been to combine the proven efficacy of an artemisinin derived anti-

malarial, artemether, with a newly discovered anti-malarial agent, benflumetol (AMMS, 1993). This is being jointly developed by the Chinese Academy of Military Medical Sciences (AMMS), Beijing, and Novartis (formerly Ciba-Geigy) in Switzerland.

For over 2,000 years the Chinese have used artemisinin (qinghaosu), an extract from the wormwood plant (*Artemisia annua*), for the treatment of malaria. Recently, it has been found to be active against chloroquine-resistant *P. falciparum*, with little toxicity. More assimilable derivatives, artemether, artesunate and arteether-have been developed and found to be similarly efficacious. Their sesquiterpene lactone structure contains an endoperoxide group that appears essential for anti-malarial activity. They comprise a unique anti-malarial drug group, markedly different in structure from all other anti malarials, and have been used both in monotherapy and in combination with other anti-malarials (Looareesuwan, 1994; Hien, 1994; Nosten *et al*, 1994). In two recent trials, one on Gambian children with cerebral malaria (Van Hensbroek *et al*, 1996) and the other on Vietnamese adults (Hien *et al*, 1996) intramuscular artemether compared favorably with quinine treatment. Both these clinical and *in vitro* studies have indicated artemisinin and its derivatives to be effective, fast acting anti-malarial drugs, with low toxicity, but marred by their relatively high recrudescence rates when used alone.

Artemether has been shown to be active against chloroquine-resistant falciparum malaria in China. It is a fast acting drug with rapid onset of its schizontocidal action on the parasite. Parasite clearance is rapid but may not be sustained because of its short half-life, leading to the high recrudescence rates (from 12% to as much as 55%: AMMS, 1991) observed with its use as a monotherapeutic agent.

Investigators in Beijing have recently discovered benflumetol, a dichlorobenzylidene derived structure and initial studies strongly suggested its effectiveness in monotherapy against malaria (AMMS, 1993). Benflumetol, in contrast to artemether, has a much slower onset of action, with a longer half-life, and it can effect a complete and sustained parasite clearance.

The possibility that improved anti-malarial efficacy could be achieved by combining artemether and benflumetol was therefore investigated.

MATERIALS AND METHODS

Patients

102 Chinese patients in Hainan Province (79 males; 23 females), aged 13-59 years (median 23) weight 35-64 kg (median 50), each with a naturally acquired, symptomatic, and previously untreated *P. falciparum* infection (asexual parasitemia >1,000 and < 200,000 µl, were selected for this noncomparative, single-center trial.

Exclusion criteria were: pregnancy; breast feeding; heavy smokers (>20 per day); infections with other *Plasmodium* species; severe CNS symptoms; hematocrit (PCV) < 20%; hypoglycemia; serious GI tract or other infections (*eg* TB); major hepatic or renal dysfunction; any prophylactic or therapeutic anti-malarial drug treatments in the preceding 4 weeks. Eighty-seven of the patients had experienced previous malarial infections between 1 and 120 months prior to the trial.

Earlier *in vivo* trial (1987-1992, data on file at AMMS) found *P. falciparum* resistant to chloroquine (30-70%) and to piperaquine (50-75%). *In vitro* studies (1991, data on file at AMMS) showed *P. falciparum* chloroquine resistance to be 98%.

Independent ethical review boards were not available in China at the time of the trial, though the combination therapy employed had been registered there. The formal approval of the local health authority was obtained before commencement of the trial. A Ciba Clinical Trial Monitor was resident on site throughout and monitored the trial according to International Good Clinical Practice (GCP) and Ciba Standard Operating Procedures. All patients were instructed on the trial objectives, methodology and the potential benefits and risks prior to their signing consent forms. Patients remained in a screened ward at the hospital in Sanya City, a transmission-free zone, for 28 days in order to (1) prevent reinfection, and (2) aid correct clinical diagnosis of any recrudescence. The patient demographic and baseline data are summarized in Table 1.

Dosage and administration

Each orally administered CGP 56697 tablet contained 20 mg artemether and 120mg benflumetol.

Table 1

Demographic and baseline data.

		n=102
Sex (M/F)	Number	79/23
Weight (kg)	Median	50
	Range	35-64
Age (years)	Median	23
	Range	13-59
Previous infection	Number	87
	If yes,	
	≤ 6 months	21
	≤ 1 year ago	39
	> 1 year ago	27
Temperature	Median	38.6
(°C)	≤ 37.5 °C	28
	> 37.5 °C	36
	≥ 39 °C	38
Parasite density (/μl)	Median	14,973
	< 5,000	21
	≥ 5,000 but<15,000	30
	≥ 15,000 but < 50,000	25
	≥ 50,000	26

Each patient received a total of 4 doses of 4 tablets each. The first dose was given following microscopic (parasitemia) diagnostic confirmation and completion of the pre-enrolment tests. Subsequent doses were then administered at 8, 24 and 48 hours. Thus, each dose comprised 80mg artemether and 480mg benflumetol, giving a total of 320mg artemether and 1,920mg benflumetol per treatment course. Each dose was given after a light meal, or 200ml water for those who were not taking food.

Pre-enrolment and trial examinations

Patients selected as suitable for trial, as defined above, underwent preenrolment investigation, which included clinical examination, X-ray, ECG and tests for blood sugar, hematology, parasitology, with concomitant urine analysis (to detect other antimalarial drug usage) and blood biochemistry assays. Axillary body temperature was taken at baseline, then every 6 hours during Week 1, and every 12 hours in the following 3 weeks.

Parasitology films were taken at baseline, then every 6 hours until negative, then daily to the end of week 1, and subsequently once each week to the end of the 4 week trial period. Parasite density was calculated as no: parasites/100 WBC × actual WBC/μl. Hematological, urine and blood biochemistry assays were made at baseline, on Day 4, and then every week. ECGs were made on enrolment, on Day 4 and Day 8.

Removal of patients from trial or analyses

Patients showing poor compliance or proving uncooperative were removed from the trial. 3 patients were not treated and removed from the trial (2 had TB and 1 absconded), reducing the total number in the trial from 105 to 102 (n=102). Evaluation of fever clearance in relation to time was only possible on evaluable patients, *ie* those with temperatures greater than 37.5°C at baseline (n=74, Table 1).

Assessment of teatment efficacy

Standard parameters were used. The primary variable was the 28 day cure rate, *ie*% patients cleared of parasitemia within 7 days of treatment onset and without subsequent recrudescence.

Secondary variables assessed were: (1) time to parasite clearance (PCT), *ie* time from first dose to total disappearance of asexual parasite forms in blood and sustained for 48 hours, (2) time to fever clearance (FCT), *ie* time from first dose until body temp ≤ 37.5°C for at least 48 hours, (3) parasite reduction % at 24 hours after initiation of treatment.

Artesunate was used as the rescue medication for any patients requiring further treatment in the course of the trial.

Statistics

An earlier clinical trial (AMMS, 1991) with CGP 56697 on 196 Chinese patients, carried out by the Chinese Academy of Military Medical Sciences gave a cure rate of 97.4 %. Calculations of a one-

Table 2

Efficacy results.

Intention-to treat	n=102
28 day cure rate	96.1% 90% CI (91.8 %, 98.7 %)
Parasite reduction at 24 hours (median)	99.4% 25th-75th percentiles; 97.9 %-100 %
Time to parasite clearance (median)	30 hours 95% CI (30 hours, 36 hours)
Time to fever clearance (median)	18 hours 95% CI (12 hours, 24 hours)

sided 95 % interval gave a lower limit of 94.7%. Statistical evaluation of the treatment efficacy therefore assumed a cure rate of at least 94.7%, with the detection of an absolute deviation of 10 % assessed as a highly likely proposition. On this basis the hypothesis of a cure rate of 94.7 % would be invalidated if the real cure rate was less than 84.7%. Application of the null hypothesis test (Ho : cure rate > 94.7%) against two alternatives (84.7% and 89.7%) established that a population size of 50-100 was sufficient to detect a deviation of 10% from 94.7%, with a power of more than 80%. On this basis the criterion for efficacy was a cure rate significantly higher than 84.7%. A 90% confidence interval using Pearson-Clopper limits was calculated for the 28 day cure and recrudescence rates. The median and percentiles with minimum and maximum values were calculated for the % parasite reduction after 24 hours. The median and means were calculated for PCT and FCT, and additionally these were calculated from first dose to first negative parasite count/first temperature recording ≤ 37.5°C.

Laboratory investigations

Blood sample assays were made at baseline and at different stages throughout the trial for: hemoglobin; hematocrit, RBC; WBC; platelets; neutrophils; lymphocytes; SGOT; SGPT; creatinine; bilirubin Urinalysis were made to detect any unauthorized use of other anti-malarials (chloroquine, sulfonamides) by patients.

RESULTS

28 day cure rate

98 of the 102 patients were cured (Table 2), a cure rate of 96.1%, which was significantly higher than the criterion for efficacy established as no less than 84.7%. Four patients (3.9%) showed recrudescence at Days 18, 24, 25 and 26 respectively, and all responded to artesunate rescue therapy. No patients had mixed infections at trial start, nor was infection by any other *Plasmodium* species (*eg P.vivax*) detected during the trial.

Parasite reduction at 24 hours

93 patients had more than 90% clearance compared to baseline at 24 hours, including 25 patients who reached complete parasite clearance in this time. Median parasite reduction at 24 hours was 99.4%.

Time to parasite clearance (PCT)

PCT ranged from 24-54 hours (median 30 hours), with a 95% confidence interval 30, 36 hours, (Kaplan-Meier method). Parasite density at baseline had a significant effect on PCT (Wilcoxon test, $p < 0.001$), the higher the density, the longer the PCT (30 hours for those < 5,000/µl and 36 hours for those ≥ 50,000/µl. The overall results for the 102 patients'PCT values are summarized in Fig 1.

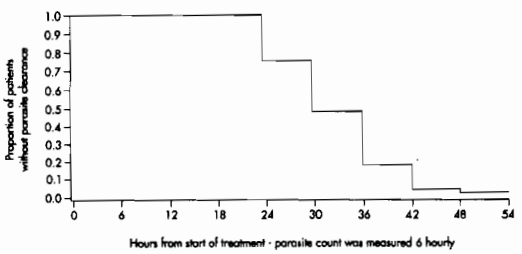

Fig 1– Time to parasite clearance (intention-to-treat, n = 102)

Time to fever clearance (FCT)

For the evaluable patients (n=74, Table 1) the median temperature at start was 39°C. The range of subsequent median temperature drops is summarized in Fig 2. Even after six hours a clear drop of 1.5°C was observed, with continued regular progression downwards thereafter. FCT ranged between 6 and 78 hours, with a median of 18 hours (95% confidence limit, 17.7, 25.1 hours). The median FCT was shorter for patients ≤ 18 years of age, or those with a previous malaria infection in the preceding 6 months.

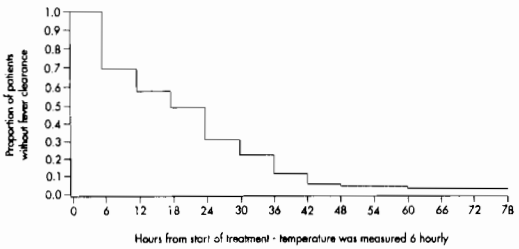

Fig 2– Time to fever clearance (evaluable patients, n = 74)

Adverse effects and safety monitoring

No adverse effects were observed, or reported by patients, during the trial. Abnormal ECGs were found in 3 patients at trial entry, in a further 2 patients on Day 4 and in one patient on Days 4 and 8 (automated ECG machine printout). ECGs were reviewed by an external cardiologist of the Chinese University of Hong Kong, and considered to be without clinical significance.

Clinical laboratory evaluations

Hemoglobin values ranged from 53-159g/l (median 118.5g/l) at baseline, with 48 patients below normal. Values decreased at Day 4, but recovered thereafter. Hematocrit values were 17-57.5% (median 39%) at baseline and also decreased slightly at Day 4, but increased to above baseline at follow-up. A similar pattern was observed for RBC values. Platelet values (39-290.10^9/l; median 127.10^9/l) were less than normal for 25 patients at baseline, but increased with treatment and 17 were above normal a follow-up. Likewise, for WBC (2.9-10.2.10^9/l; median 4.9.10^9/l), with 22 patients below normal at baseline, but rising to normal or above normal during the trial. Similarly, with the lymphocyte and neutrophil counts. At baseline 6 patients had greater than normal SGOT and 3 patients had above normal SGPT, but these reduced with treatment. None of the total bilirubin levels were above normal throughout the trial, with most below the lower limit of normal (1.7 mmol/l). 8 patients had above normal creatinine at baseline, 7 at Days 4 and 13 at follow-up.

DISCUSSION

The open non-comparative trial reported in this communication confirms and extends the earlier studies performed in China by AMMS of the drug combination therapy of artemether and benflumetol (CGP 56697) combining the advantages of both compounds simultaneously in the treatment of naturally acquired, uncomplicated, acute falciparum malaria.

A short course of four doses given over 48 hours achieved a final cure rate of 96.1% over the 28 day trial period. 25 out of the 102 patients reached complete parasite clearance within 24 hours of treatment onset. Ninety-three patients had more than 90% parasite clearance at 24 hours. These results clearly confirm the rapid onset of CGP 56697 schizontocidal activity. The recrudescence rate (3.9%) was low.

Parasite clearance times (PCTs) ranged from 24 to 54 hours, with a median value of 30 hours; the progressive elimination of parasite infection is illustrated in Fig 1.

The time to fever clearance (FCT) was also rapid (median 18 hours), as shown in Fig 2.

No adverse effects from use of CGP 56697 were observed or reported, and the various biochemical assays at baseline and throughout the trial were not at variance with normal expectations.

The majority of patients treated had a history of previous infection with malaria (Table 1) and had, therefore, acquired some degree of immunity, but insufficient to protect them against subsequent reinfection.

The trial confirms the efficacy and safety of this combination therapy of artemether with benflumetol

for semi-immune adults living in a malaria endemic region in China, where there was known chloroquine resistance.

These positive findings have heralded a series of more extensive comparative trials of this CGP 56697 combination therapy in other endemic malaria areas (Africa, India, Thailand), which are currently ongoing and will be reported soon.

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