The Lancet · Saturday 7 August 1982

ANTIMALARIAL ACTIVITY OF MEFLOQUINE AND QINGHAOSU

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Summary In a chloroquine-resistant *Plasmodium falciparum* endemic area of Hainan Island, China, 1·0 g oral mefloquine produced a radical cure in 47 of 48 semi-immune patients. A comparison between patients treated with mefloquine and with oral qinghaosu showed a more rapid clearance of parasitaemia with qinghaosu (68·2±21·4 h *vs* 103·1±18·0 h) and a greater inhibition of in-vivo trophozoite development. An advantage of mefloquine is the effectiveness of a single oral dose, whereas the advantages of qinghaosu are the speed of onset of action and inhibitory effect on parasite maturation.

Introduction

THE emergence and distribution of chloroquine-resistant falciparum malaria is widely known and well documented throughout most of the world. Recently chloroquine resistance has been found in China, especially Hainan Island.[1,2] Pyrimethamine-sulfadoxine ('Fansidar') alone, or preferably with quinine, is effective for the treatment of chloroquine-resistant and chloroquine-sensitive *Plasmodium falciparum*,[3,4] but recently resistance to pyrimethamine-sulfadoxine and even to quinine has been reported in South-East Asia.[5,6]

New antimalarial drugs are urgently needed: in the early 1970s the U.S. Army Antimalarial Drug Research Programme discovered and developed mefloquine (fig. 1), a long-acting quinine analogue with a half-life of 6 to 22 days.[7] Mefloquine is curative against chloroquine-resistant falciparum malaria[4,8] and against vivax malaria (Prof. T. Harinasuta, Prof. C. V. Uylangco, unpublished). It also actively suppresses both falciparum and vivax infections.[9]

In China a crude extract of a medicinal herb (*Artemisia annua L*; fig. 2) has been in use for 2000 years and Chinese

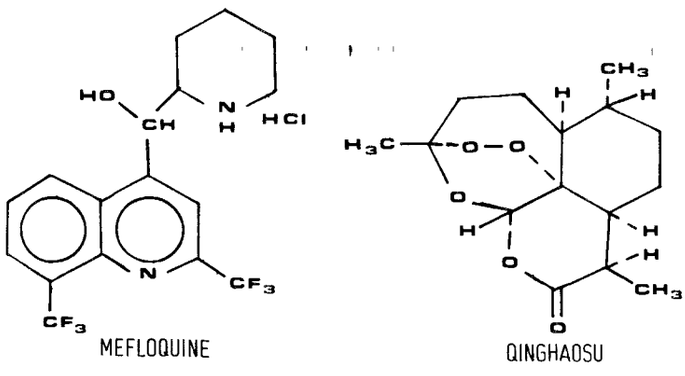

Fig. 1—Mefloquine, a 4-quinoline methanol, and qinghaosu, a sesquiterpene lactone with a peroxy group.

Fig. 2—*Artemisia annua*.

writings indicate that the extract was used for treating malaria. This antimalarial activity was rediscovered in 1971 and since then a purer crystalline extract named qinghaosu (fig. 1), which has a short half-life (hours), has been studied in animal malaria models and in man.[10] It has a rapid onset of action and low toxicity and is effective against vivax malaria as well as chloroquine-sensitive and chloroquine-resistant falciparum malaria.[10] This study was undertaken to document further the clinical effect of mefloquine in the treatment of falciparum malaria and to make a preliminary qualitative comparison of the acute effect of mefloquine and qinghaosu on the in-vivo development of asexual parasites of *P. falciparum*.

Patients and Methods

The study was carried out between October, 1980, and March, 1981, in the Dong Fang district hospital on the Western tip of Hainan Island, off the southern coast of China. The predominant malaria species on the island is *P. falciparum* (unpublished) and the level of chloroquine resistance is high.[2]

Patients were selected from those attending the hospital outpatient department who were febrile and had clinical symptoms of malaria, positive blood smear for *P. falciparum* asexual parasites with a count greater than 1000/μl blood or 5000/μl blood if they had been ill for longer than 5 days, and no previous treatment for the present illness. Patients under 8 years and over 60 years, pregnant women, and those with previous or present cardiac, hepatic, or renal disease were excluded. The details of the study were explained to the patients, and verbal consent to participate was obtained.

Study patients were admitted to hospital for 7 days and returned for follow-up examinations 14, 21, 28, 40, 50, and 60 days after drug administration. A detailed record of signs and symptoms was kept: the temperature was measured every 4 h until the patient was afebrile for 24 h, and then once a day. Thick films were examined every 12 h in hospital, and at follow-up visits; and if no sexual forms were seen, parasitaemia was considered cleared. Quantitative parasite counts with a thin smear were carried out for those with positive thick films. Routine laboratory tests on blood and urine for haematological, liver, and renal function were carried out regularly, and the urine was tested for chloroquine.[11] Treatments were evaluated according to the World Health Organisation in-vivo criteria suggested for determining chloroquine resistance.[12] An S response (radical cure) is defined as clearance of asexual parasites by day 6 with no reappearance of parasites by day 28, an RI response as parasitaemia clearing by day 6 but reappearing before day 28, an RII response as parasitaemia falling to below 25% of pretreatment level but not clearing by day 6, and an RIII response as parasitaemia not falling below 25% of pretreatment level or increasing before day 6. Mefloquine was administered in a single oral dose under supervision; adults received 1·0 g, children 11–15 years 0·75 g, and children 8–10 years 0·5 g.

To compare the effects of mefloquine and qinghaosu on *P. falciparum* asexual parasite clearance and development, patients with a parasite count between 10 000 and 100 000/μl blood were studied. 10 patients received 1·0 g mefloquine; 10 received oral qinghaosu, 1·0 g initially and 1·0 g 24 h later; 10 received intramuscular qinghaosu in a water suspension, 0·6 g initially, 0·3 g after 24 h, and 0·3 g after 48 h; and 10 received intramuscular quinine dihydrochloride, 0·5 g every 8 h for 3 days. The drugs were given when the blood film showed trophozoites at the tiny-ring stage, which corresponds to the first 6 h after penetration into the red cells by the parasites.

For this part of the study blood films were examined every 4 h to monitor clearance of parasitaemia and to observe maturation of the tiny-ring trophozoite through the small-ring form to the large-ring form.[13] Jiang et al. have shown in patients examined with thick blood films taken every 2 h, and in a monkey model (unpublished), that the asexual trophozoite of *P. falciparum* passes successively through five stages of development: a tiny-ring form, in which the width of the cytoplasm band is less than half the diameter of the nucleus (appears within the first 6 h of the asexual cycle); a small-ring form in which the width of the cytoplasm band is greater than half of the diameter but less than the diameter of the nucleus (6–16 h); a large-ring form, in which the greatest width of the cytoplasm band is equal to or greater than the diameter of the nucleus (16–26 h); the late trophozoite form (26–36 h) in which the cytoplasm is a blue mass larger than the nucleus and pigment is apparent, but the nucleus although larger remains undivided; and the schizont (36–48 h) in which the trophozoite nucleus has divided. The stages of parasite development between 26 and 48 h are not usually seen in patients, but they can be seen clearly in splenectomised rhesus monkeys with human blood replacement (Jiang et al., unpublished).

Results

Mefloquine Clinical Study

We treated 51 patients, 31 male and 20 female, aged 8–53 years (mean 19 years): 31 patients were older than 15 years, and 20 patients 8 to 15 years old. 3 patients were from non-malarious regions and 48 patients were living in an endemic malaria area. 49 patients had negative urine tests for chloroquine, and 2 patients had received chloroquine elsewhere as the initial treatment but had failed to respond. On admission all patients were febrile (axillary temperature >37·5°C), and 47 patients had temperatures ⩾38·1°C. 15 patients had nausea and vomiting, 4 had diarrhoea, 49 had headache, and 18 had vertigo. Hepatomegaly and splenomegaly were present in 40 and 43 patients, respectively. The mean asexual parasite count of *P. falciparum* was 47 829/μl blood (range, 1748–234 175/μl; <10 000/μl in 19·6% of patients; 10 000–50 000/μl in 49·1%; 51 000–100 000/μl in 17·6%; and >100 000/μl in 13·7%).

47 of 48 inhabitants of the endemic area had an S response (radical cure) and 1 patient had an RI response with recrudescence on day 28. Of the 3 non-immune patients 1, who had not responded to chloroquine, had an RI response, and another had an RII response. The 3rd non-immune patient had an initial parasite count of 234 175/μl, and parasite counts were carried out hourly after 1·0 g mefloquine. Since the tiny-ring forms continued to develop into later ring forms the patient was given a qinghaosu derivative intravenously, because of concern about the development of cerebral malaria. He responded rapidly to the qinghaosu derivative, and parasitaemia cleared (RI) but recrudesced on day 21. The overall cure rate in 51 patients was 92·2%, and in 48 semi-immune patients 97·9%. 3 patients had initial mixed infections with both *P. falciparum* and *P. vivax*; they responded to the acute treatment but 1 patient relapsed with *P. vivax* on day 21.

Body temperature returned to normal in a mean of 29·2±18·0 h (SD) and the blood film became negative after a mean 92·9±22·8 h. 46 patients returned for all follow-up examinations until day 60 with 1 patient having a positive blood film on day 50. 1 patient had a blood film positive for *P. vivax* on day 21, and 11 patients were positive for *P. vivax* between day 40 and day 60. 46 of the 48 inhabitants of the endemic area were followed long-term but returned to malarious areas after hospital discharge. The patient who had a positive *P. falciparum* film on day 50 could have recrudesced but he had returned to the endemic area and could have been reinfected. Another patient who stayed in town (non-malarious) recrudesced on day 28.

Mefloquine/Qinghaosu Comparison

The times taken by mefloquine and quinine to eliminate parasitaemia and to lower fever were not significantly different (table I). Qinghaosu orally or intramuscularly cleared parasitaemia significantly more rapidly than mefloquine or quinine, with the oral route being more effective (table I). The times to become afebrile with oral and intramuscular qinghaosu were similar to that for mefloquine.

TABLE I—EFFECT OF MEFLOQUINE, QINGHAOSU, AND QUININE ON TEMPERATURE AND PARASITAEMIA IN FALCIPARUM MALARIA

Group*	Parasite clearance time (h)		Fever clearance time (h)	
	Mean±SD	p†	Mean±SD	p†
Oral mefloquine	103·1±18·0	..	30·6±18·7	..
Intramuscular quinine	104·0±13·2	NS	42·2±25·2	NS
Intramuscular qinghaosu‡	78·7±19·4	<0·05	33·7±21·2	NS
Oral qinghaosu	68·2±21·4	<0·01	22·3±10·4	NS

*n = 10 in each group.
†All p values for comparison with the corresponding mefloquine result.
‡Water suspension.
NS = Not significant.

There is rapid parasite clearance with oral qinghaosu and progressively less rapid clearance with intramuscular qinghaosu, mefloquine, and quinine. The parasite count fell more than 95% in 20 h with oral qinghaosu, in 24 h with intramuscular qinghaosu, in 28 h with mefloquine, and in 36 h with quinine.

The rate of parasite clearance and effect on parasite development for 8 patients treated with mefloquine and 8 treated with oral qinghaosu are shown in fig. 3 and table II. The slower onset of action of mefloquine may result in an increase in parasitaemia during the first few hours of treatment if it is given at the tiny-ring stage. 16 h after mefloquine given at the tiny-ring stage (0 h) 73·7% of the parasites had developed to the small-ring stage, and 25·6% to the large-ring stage. 16 h after qinghaosu the parasite count had fallen greatly and 57·1% of the remaining parasites were at the small-ring stage, 42·9% were still tiny-ring forms, and there were no large-ring forms. The faster rate of parasite clearance and greater inhibition of parasite development by qinghaosu was also apparent 24 h after drugs (fig. 3 and table II). 24–28 h after mefloquine the trophozoites developed into the large-ring form, as happened in an untreated control, whereas oral qinghaosu arrested trophozoite development at the small-ring stage.

TABLE II—PERCENTAGE DISTRIBUTION OF DIFFERENT STAGES OF PARASITE DEVELOPMENT AFTER MEFLOQUINE AND ORAL QINGHAOSU*

Time since drug (h)	Mefloquine			Qinghaosu		
	Tiny	Small	Large	Tiny	Small	Large
0	66·4	27·8	5·8	67·8	30·9	1·3
8	17·0	82·1	0·9	52·2	47·4	0·5
16	0·7	73·7	25·6	42·9†	57·1†	0†
24	0·4	51·3	48·3	26·5‡	73·5‡	0‡

*For each of the 16 patients a thick blood smear was examined at each time, and stage of development of 200 asexual parasites was assessed. Numbers of tiny, small, and large ring forms were then calculated as a percentage of the total number of parasites examined for the 8 patients in each treatment group at each time interval.
†Only 7 patients included since 1 had a negative smear.
‡Only 2 patients included since 6 patients had negative smears.

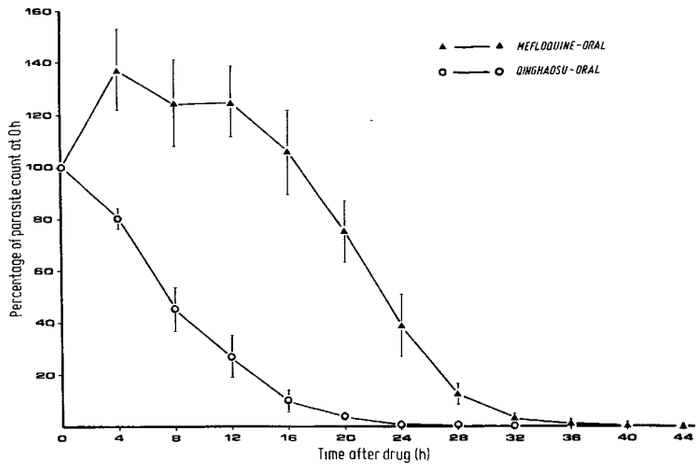

Fig. 3—Effect of mefloquine and oral qinghaosu on rate of *P. falciparum* parasite disappearance.

Drugs were given at the tiny-ring stage (0 h), equivalent to 4–6 h of parasite development within the red cell. The absolute parasite count at 0 h is taken as 100% and later parasite counts expressed as a percentage of the 0 h count in each patient. Mean±SEM of these relative counts for group of 8 semi-immune patients treated with each drug was then plotted against time.

Side-effects attributed to mefloquine were nausea and vomiting in 5 of the 51 patients, diarrhoea in 2, and vertigo in 1 patient. Results of routine tests of haematology and renal function were unchanged during mefloquine treatment. Serum alanine aminotransferase levels rose slightly in 6 patients but returned to normal by day 15.

Discussion

In this study mefloquine was effective against *P. falciparum* with a radical cure rate of 92·2%; this result compares favourably with earlier studies in volunteers infected with chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*,[14] where 10 of 12 and 8 of 8 subjects were cured with 1·0 g and 1·5 g, respectively. In patients naturally infected in Thailand, 94%[15] and 100% (of 37)[8] were cured with 1·5 g. Of the 4 RI, RII, and RIII responses in our study, 3 were in non-immune patients and, since the equivalent of only 1·0 g of mefloquine was given, it is possible that the higher dose (1·5 g) used in other studies would be preferable in non-immune patients.

The suppressive effect of mefloquine was not directly tested but after patients had returned to transmission areas only 2 patients recrudesced or were reinfected; this finding suggests that the drug is an effective prophylactic agent. This observation has been made before[8] and has been confirmed in prospective chemosuppressive trials.[9] The prophylactic effect of qinghaosu has not yet been studied.

Qinghaosu cleared parasitaemia more rapidly than mefloquine or intramuscular quinine; oral qinghaosu acted more quickly than intramuscular qinghaosu-water suspension. Mefloquine and qinghaosu clearly destroy *P. falciparum* asexual parasites, but qinghaosu acts more quickly than mefloquine to prevent the further development of the early trophozoite. These characteristics of qinghaosu may make it useful in patients with high parasite counts, since the rapid reduction in parasite count and more complete arrest of parasite development could reduce the risk of cerebral malaria.

Other studies[2,10] found a lower radical-cure rate for oral qinghaosu (20%) than for intramuscular qinghaosu (>90%).

Although pharmacokinetic data on qinghaosu in human beings are not yet available, this low radical-cure rate is probably related to the short half-life of the drug when given orally (4 h in mice),[10] since some asexual parasites will escape destruction and will complete the maturation cycle to proliferate. This is true for quinine, with a half-life of about 10 h, which is poorly effective in achieving a radical cure when used alone and in a short course. The long-acting antimalarials such as mefloquine remain in the blood at a concentration high enough to destroy the successive cycles of surviving trophozoites until none remain viable. The convenience of a single oral dose of mefloquine is an advantage, but the speed of action and more complete inhibition of parasite development with qinghaosu are also distinct advantages. The intramuscular injection of qinghaosu is very effective, but a 3-day course is necessary: this regimen therefore has disadvantages in terms of convenience, compliance, and comfort.

The appearance of *P. vivax* in several patients after treatment of *P. falciparum* malaria with mefloquine indicates that mefloquine does not affect the intrahepatic stage of *P. vivax* and, as with chloroquine, primaquine must be given to effect a radical cure in vivax malaria.

An antimalarial with the combined advantages of mefloquine and qinghaosu would be very valuable in the treatment of acute falciparum malaria, but the combination of a short-acting and a long-acting drug, although very effective therapeutically, does not avoid the potential problem of resistance developing against the long-acting component.

This work was supported by the Roche Far East Research Foundation (K. A.) and the Kevin Hsu Research Fund (Y. C. K.). Clinical support facilities provided by the Dong Fang District hospital are gratefully acknowledged.

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BLISTERS, PRURITUS, AND FEVER AFTER BITES BY THE ARABIAN TICK ORNITHODOROS (ALECTOROBIUS) MUESEBECKI

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Summary A biologist was bitten by *Ornithodoros (Alectorobius) muesebecki* Hoogstraal, an endemic tick parasite of nesting and resting marine birds on islands in eastern Arabia. Irritating bullae developed and for four months he experienced intermittent inflammation and irritation. Two years earlier, after being bitten by the same tick species on a different island, he had experienced only irritation lasting no more than a fortnight. Petroleum-industry labourers on another island were admitted to hospital for about two weeks with bullae at numerous bite sites, intense pruritus, headache, and fever. Zirqa virus (Bunyaviridae, *Nairovirus*) has been isolated from *O. (A.) muesebecki* samples from Abu Dhabi. The role of Zirqa virus and/or of salivary toxins in producing irritation and illness, as well as individual sensitivity to the tick and the seasonal dynamics of toxicity or infectivity, should be investigated.

Introduction

ORNITHOLOGISTS and other visitors to breeding and resting sites of marine birds in the Arabian Sea, Gulf of Oman, and Arabian Gulf expose themselves to bites by the tick *Ornithodoros (Alectorobius) muesebecki* Hoogstraal. The bites are followed by toxin or infection induced discomfort or illness.[1,2] The variety of poorly defined sequelae accompanying *O. (A.) muesebecki* bites require careful investigation.

Case-report

From Sept. 21 to 27, 1981, one of us (M. D. G.) camped alone near an abandoned nesting site (apparently of the crested tern, *Sterna berghii*) on Al Hallaniyah island, the largest of the Kuria Muria group (Sultanate of Oman), in the northern Arabian Sea. After sunset, particularly on the first evening, he was overrun by *O. (A.) muesebecki* and counted about 20 bites, chiefly on the ankles but also on the soles of the feet and on the legs, waist, trunk, neck, and hands and face. The ticks rapidly climbed upward and over and under clothing and bedding. Within a few hours the bite sites were inflamed and caused irritation. Areas on the ankles from which ticks were hurriedly removed while becoming engorged became small bullae within 24 h; these were not caused by sepsis or scratching. The accompanying figure shows bullae on the leg on Sept. 28, one week after the first bites. The lower bullae were subsiding but still caused irritation; the upper bulla was redeveloping after having subsided. The bullae were then pricked with a needle to release the serous fluid. However, the perforations healed quickly and the treatment had to be repeated morning and evening. The wounds were smeared with 'Cetavlex' (cetrimide 0·5%) cream and protected from dust by binding with 'Micropore' surgical tape over cotton wool. The bullae took two weeks to dry and to heal. Slight inflammation and irritation recurred occasionally during the next four months.

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