

Atovaquone and Proguanil Hydrochloride Compared with Chloroquine or Pyrimethamine/Sulfadoxine for Treatment of Acute *Plasmodium falciparum* Malaria in Peru

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The efficacy and safety of a fixed-dose combination of atovaquone and proguanil hydrochloride (Malarone™) were compared with chloroquine or pyrimethamine/sulfadoxine in patients with acute falciparum malaria in northern Peru. Patients were initially randomized to receive 1,000 mg atovaquone and 400 mg proguanil hydrochloride daily for 3 days (n=15) or 1,500 mg chloroquine (base) over a 3 day period (n=14) (phase 1). The cure rate with chloroquine was lower than expected and patients were subsequently randomized to receive a single dose of 75 mg pyrimethamine and 1,500 mg sulfadoxine (n=9) or atovaquone/proguanil as before (n=5) (phase 2). In phase 1, atovaquone/proguanil was significantly more effective than chloroquine (cure rate 100% [14/14] versus 8% [1/13], $P < 0.0001$). In phase 2, atovaquone/proguanil and pyrimethamine/sulfadoxine were both highly effective (cure rates 100% [5/5] and 100% [7/7]). There were no significant differences between treatment groups in parasite or fever clearance times. Adverse events were typical of malarial symptoms and did not differ significantly between groups. Overall efficacy of atovaquone/proguanil was 100% for treatment of acute falciparum malaria in a region with a high prevalence of chloroquine resistance.

Key Words: Malarone, atovaquone, proguanil, chloroquine resistance, Peru.

Drug resistant strains of *Plasmodium falciparum* pose an increasing therapeutic dilemma. Chloroquine resistance is widespread in South America, sub-Saharan Africa and Southeast Asia [1]. In many parts of the world, *P. falciparum* has also developed resistance to pyrimethamine/sulfadoxine, quinine, mefloquine, and halofantrine [2]. Safe and effective new antimalarial drugs are needed.

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Atovaquone is a hydroxynaphthoquinone that inhibits parasite mitochondrial electron transport [3]. Proguanil is an isopropylbiguanide that inhibits plasmodial dihydrofolate reductase via its metabolite cycloguanil [4]. The combination of atovaquone and proguanil has synergistic blood schizonticidal activity *in vitro* [5] and enhanced efficacy when used to treat drug-resistant strains of *P. falciparum*. In patients with acute, uncomplicated falciparum malaria, efficacy was 66% in patients treated with atovaquone alone, 8% in patients treated with proguanil alone, and 99% in patients treated with atovaquone and proguanil in combination [6]. Atovaquone/proguanil has also been shown to be highly effective and extremely well tolerated for prophylaxis of falciparum malaria [7-10].

Previous clinical trials evaluating atovaquone/proguanil for treatment of malaria used individual tablets of each component. In the clinical trial presented here,

a fixed-dose combination tablet of atovaquone and proguanil hydrochloride (Malarone™) was evaluated for treatment of acute, uncomplicated falciparum malaria in adolescents and adults in northern Peru.

Materials and Methods

This study was conducted from June, 1995, to May, 1996, in Piura, a medium-sized city in northern Peru where malaria is endemic. Patients were lifelong residents of the area and were considered semi-immune. Patients with acute, uncomplicated falciparum malaria were eligible for the study if they were 12 to 65 years of age and had an initial parasite count between 1,000 and 200,000 parasites/ μ L blood. Patients were excluded if they had severe malaria, significant concomitant disease or a mixed plasmodial infection, or if they were pregnant, lactating or unable to tolerate oral therapy.

Study design. The trial began as an open-label, randomized comparison of atovaquone/proguanil versus chloroquine. After 29 patients had been enrolled, the cure rate with chloroquine was 8%, and the protocol was amended to replace chloroquine with pyrimethamine/sulfadoxine for subsequent patients. However, patient recruitment declined and the second phase of the trial ended after an additional 14 patients were enrolled.

Treatment assignment. In Phase 1, patients were randomly assigned to receive either 4 tablets, each containing 250 mg atovaquone and 100 mg proguanil hydrochloride, once daily for 3 days (total daily dose 1,000 mg atovaquone and 400 mg proguanil hydrochloride) or chloroquine sulfate, 150 mg (base) per tablet (total dosage 1,500 mg during 3 d; 600 mg followed by 300 mg at 6, 24, and 48 h later). Food or soya milk was given 45 minutes before dosing to increase the bioavailability of atovaquone [11].

In Phase 2, patients were randomly assigned to receive either atovaquone/proguanil, as above, or pyrimethamine/sulfadoxine administered as a single dose

of 3 tablets, each containing 25 mg pyrimethamine and 500 mg sulfadoxine (total dose 75 mg pyrimethamine and 1,500 mg sulfadoxine).

All antimalarial drugs were given orally under the supervision of the investigators. Patients who vomited within 1 hour of receiving the study drug were given another dose.

Clinical and laboratory assessments. Clinical examinations were done at least once daily for the first 7 days, and on days 14, 21, and 28 after the start of treatment. Patients were queried from a standard list of 16 symptoms commonly associated with malaria, and asked open-ended questions about other symptoms. Body temperature was measured every 4 h until normal for 2 d.

Thick and thin blood films were prepared every 6 h for the first 7 days or until 2 films were free of asexual parasites. Films were then prepared weekly until day 28. Parasite counts were determined from Giemsa-stained blood films as the number of asexual parasites per 1,000 red blood cells (thin film) or per 200 white blood cells (thick film) and expressed as parasites per microliter of peripheral blood. Blood films were considered to be negative when examination of 200 oil-immersion fields on a thick film showed no asexual parasites.

Blood was obtained for routine hematology and clinical chemistry studies before treatment and on study days 3, 7, 14, and 28.

Efficacy endpoints. The primary efficacy endpoint was the 28-day cure rate. The response to treatment was based on the WHO classification [12]: S = parasite clearance within 7 days without recrudescence up to day 28; RI = parasite clearance within 7 days, followed by recrudescence within 28 d; RII = marked reduction of parasitemia, but without clearance in 7 days; RIII = no significant reduction of parasitemia during the first 48 h. Patients who withdrew from the study or were not followed for 28 days were excluded from analysis of 28-day cure rates. Cure rates were calculated as $S/(S + RI + RII + RIII)$.

Table 1. Therapeutic response at day 28 evaluation

	Phase 1		Phase 2	
	Atovaquone/ proguanil (n = 15)	Chloroquine (n = 14)	Atovaquone/ proguanil (n = 5)	Pyrimethamine/ sulfadoxine (n = 9)
No. of evaluable patients	14	13	5	7
No. (%) of patients cured	14 (100)	1 (8)	5 (100)	7 (100)
Fever clearance time (h)				
Median	46	48	40	44
Mean (SD)	42.9 (20.5)	48.0 (16.4)	38.4 (18.9)	48.0 (13.0)
Range	8-92	20-72	20-26	28-72
Parasite clearance time* (h)				
Median	57	48	42	42
Mean (SD)	55.7 (10.6)	58.7 (25.8)	44.4 (6.8)	38.0 (14.6)
Range	36-78	24-96	36-54	24-28

* Parasite clearance times exclude 5 patients who did not clear parasitemia (RII response) after treatment with chloroquine.

Table 2. Adverse events occurring in more than 1 patient in any treatment group*

	Atovaquone/ proguanil (n = 20)	Chloroquine (n = 14)	Pyrimethamine/ sulfadoxine (n = 9)
Headache	2 (10)	6 (43)	2 (22)
Nausea	5 (25)	1 (7)	1 (11)
Vomiting	7 (35)	0	2 (22)
Abdominal pain	5 (25)	2 (14)	2 (22)
Insomnia	3 (15)	2 (14)	4 (44)
Dizziness	1 (5)	4 (29)	0
Weakness	0	5 (36)	0
Pruritus	0	3 (21)	1 (11)
Palpitations	0	3 (21)	0
Chills/rigors	1 (5)	2 (14)	0
Anorexia	2 (10)	1 (7)	0

* Values expressed as number (%) of patients with an adverse event.

Fever clearance time (FCT) and parasite clearance time (PCT) were secondary efficacy endpoints. For patients with fever at baseline, FCT was calculated from initiation of treatment until the body temperature had decreased to 37.2°C, and remained less than 37.2°C for at least 24 hours. For patients who cleared parasitemia, PCT was calculated from initiation of treatment until peripheral blood smears were first negative for asexual parasites.

Safety analysis. Data from all study participants were used in the safety analysis. Adverse events were defined as any clinical finding that first occurred or increased in intensity within 10 days of treatment initiation.

Statistical analysis. Cure rates for phase 1 were compared using Yates' corrected chi-squared analysis; cure rates for phase 2 were not compared statistically. The MannWhitney U test was used to calculate differences in medians for fever and parasite clearance times and hematology and biochemistry test results. A difference between medians was considered statistically significant if $P < 0.05$.

The study was conducted at Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru and study house at Piura, Peru. The protocol was reviewed by the Institutional Review Board of the Universidad Peruana Cayetano Heredia. All patients or their guardians gave written informed consent.

Results

Forty-three Mestizo patients between 15 and 65 years of age participated in the study. In phase 1, 14 of 15 patients treated with atovaquone/proguanil and 13 of 14 patients treated with chloroquine completed the study. One patient in the atovaquone/proguanil group had a seizure on day 3 of treatment and was withdrawn and transferred for further evaluation, which revealed a past history of seizures and a serum sodium concentration of 115 mEq/L. One patient in the chloroquine group was withdrawn because, on

day 2, he received a disallowed drug for a urinary tract infection (cotrimoxazole, which also has antimalarial activity).

In phase 2, all 5 patients treated with atovaquone/proguanil and 7 of 9 patients treated with pyrimethamine/sulfadoxine completed the study. Two patients in the pyrimethamine/sulfadoxine group withdrew consent on day 7 when they were asymptomatic.

Treatment groups did not differ significantly with respect to baseline demographic, clinical or laboratory characteristics. Laboratory values were typical of patients with acute malaria, with mild anemia (hemoglobin 75-118 g/L) and thrombocytopenia (platelet count $85-137 \times 10^9/L$) in approximately half the patients. The geometric mean initial parasite count in the 4 treatment groups ranged from 3,112/ μL to 6,688/ μL .

Efficacy. The therapeutic responses at day 28 are summarized in Table 1. In phase 1, 100% of evaluable patients in the atovaquone/proguanil group and 8% of evaluable patients in the chloroquine group were cured. This difference was highly significant ($P < 0.0001$). Of the 12 patients not cured after treatment with chloroquine, 5 did not clear parasitemia (RII response) and 7 had recrudescence parasitemia between days 6 and 20 (RI response). In phase 2, 100% of patients in the atovaquone/proguanil group and 100% of evaluable patients in the pyrimethamine/sulfadoxine groups were cured. There were no significant differences in parasite clearance times or fever clearance times between groups in either phase of the study.

Safety. Most adverse events reported were common symptoms of malaria and were also reported as pre-treatment' symptoms. The most frequently reported adverse events are shown in Table 2. The frequency of adverse events did not differ significantly between groups, although there was a trend toward more vomiting and abdominal pain in patients treated with atovaquone/proguanil and more non-specific events (headache, weakness, dizziness) in patients treated with chloroquine.

One severe adverse event occurred; a patient in the atovaquone/proguanil group had generalized seizures on day 3. Subsequent evaluation revealed

hyponatremia, not a past history of seizures. The seizures were attributed to hyponatremia, and not to treatment, and the patient was evaluated for an endocrine disorder.

Laboratory findings during and after treatment did not differ significantly between treatment groups.

Discussion

A fixed-dose combination of atovaquone and proguanil hydrochloride, given once daily for 3 d, was significantly more effective than the standard 3-day regimen of chloroquine for treatment of falciparum malaria in northern Peru (cure rate 100% versus 8%, respectively).

Data on a limited number of patients suggest that pyrimethamine/sulfadoxine is also effective for treatment for acute uncomplicated falciparum malaria in northern Peru. The high incidence of chloroquine-resistant malaria found in this study was unexpected. Chloroquine is the standard treatment for falciparum malaria in the Piura region and was thought to be about 70% effective when the study began [13].

The high efficacy of atovaquone/proguanil in the present study is consistent with the response to this regimen (administered as separate tablets of atovaquone and proguanil hydrochloride) against *P. falciparum* (including multidrug-resistant strains) in previous clinical trials in South America, Africa, and Asia. For example, atovaquone/proguanil was significantly more effective than amodiaquine in Gabon [14], mefloquine in Thailand [15], and chloroquine or chloroquine plus pyrimethamine/sulfadoxine in the Philippines [16], and had efficacy similar to quinine plus tetracycline in Brazil [17], halofantrine in Kenya [18], and pyrimethamine/sulfadoxine in Zambia [19]. The overall efficacy in 521 patients treated with the recommended dose of atovaquone/proguanil was 99% [6].

The mean parasite clearance times (44-56 h) and fever clearance times (38-43 h) observed in the atovaquone/proguanil groups in this study were similar to the range observed for atovaquone/proguanil in other clinical trials (47 to 72 h for parasite clearance time and 19 to 61 h for fever clearance time) [6].

The fixed-dose combination of atovaquone/proguanil was generally well tolerated. Most of the adverse events reported by patients were common manifestations of malaria and were also reported as pre-treatment symptoms. Although there were no significant differences between the treatment groups in the frequency of adverse events, caution should be used in interpreting these results because of the small number of patients per group. The one serious adverse event that occurred during the study (seizures) was not attributed to treatment, but rather to the patient's hyponatremia.

Although atovaquone/proguanil is safe and highly effective, atovaquone is expensive to manufacture, and atovaquone/proguanil thus costs more than most other drugs used to treat malaria. In countries with limited financial resources, alternative drugs such as sulfadoxine/pyrimethamine are, therefore, more appropriate for treatment of chloroquine-resistant malaria. However, if resistance to sulfadoxine/pyrimethamine [20, 21] and mefloquine [22, 23] continues to spread, atovaquone/proguanil may provide an important alternative for patients infected with multi-drug-resistant strains of *P. falciparum*.

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