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CLINICAL STUDIES OF ATOVAQUONE, ALONE OR IN COMBINATION WITH OTHER ANTIMALARIAL DRUGS, FOR TREATMENT OF ACUTE UNCOMPLICATED MALARIA IN THAILAND

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Abstract. The therapy of *Plasmodium falciparum* malaria continues to be a problem in many parts of Southeast Asia because of multidrug resistance to nearly all existing antimalarial drugs. Atovaquone is a novel hydroxynaphthoquinone with broad spectrum anti-protozoal activity. We recently evaluated the antimalarial activity of atovaquone in a series of dose-ranging studies in 317 patients with malaria at the Bangkok Hospital for Tropical Diseases. Originally, the drug was administered alone. Using atovaquone alone resulted in satisfactory, initial clinical responses in all patients; the mean parasite and fever clearance times were 62 and 53 hr, respectively. However, irrespective of the duration of therapy, overall cure rates were approximately 67%. In vitro sensitivity studies on parasites taken from patients prior to treatment and at the time of recrudescence showed a marked decrease in susceptibility to atovaquone in the recrudescent parasites. To improve cure rates, atovaquone was administered in combination with other drugs with antimalarial activity. Proguanil and tetracycline were chosen due to laboratory evidence of potentiation; doxycycline was selected because it has a longer half-life than tetracycline. Although pyrimethamine did not show laboratory evidence of potentiation with atovaquone, it was chosen as an alternative inhibitor of dihydrofolic acid reductase with a longer half-life than proguanil. The clinical studies with these drug combinations confirmed the laboratory results with marked improvement in cure rates for proguanil, tetracycline, and doxycycline; pyrimethamine showed only minimal improvement. Proguanil was subsequently selected as the preferred drug partner because of its long record of safety and the ability to use the drug in pregnant women and children. Of the 104 patients with falciparum malaria treated with atovaquone plus proguanil for 3–7 days, 101 were cured and had virtually no adverse side effects. The combination of atovaquone and proguanil also was effective in eliminating erythrocytic forms of *P. vivax*, but parasitemia recurred in most patients.

Atovaquone is a new antimalarial drug being developed by The Wellcome Foundation Ltd. (Beckenham, United Kingdom) for treatment of multidrug-resistant falciparum malaria. The drug is a novel hydroxynaphthoquinone, a class of drugs with known antimalarial activity since the 1940s.[1] Previous members of the class, however, showed only limited activity in humans when administered orally, and none progressed to clinical use. Atovaquone is the result of intensive synthetic efforts aimed at producing a metabolically stable compound with optimum activity against *Plasmodium falciparum* in vitro.[2] Although initially developed because of its antimalarial activity, atovaquone has broad spectrum antiparasitic activity and is presently used for treatment of opportunistic infections in patients with acquired immunodeficiency syndrome.[3,4]

Early dose-ranging studies of atovaquone for treatment of mild *P. falciparum* malaria in 10 patients in the United Kingdom showed that single doses of 500 mg uniformly resulted in a prompt clinical response with elimination of symptoms and asexual parasites from blood films (Chiodini PL, unpublished data). At this dose level, however, most patients suffered recrudescent infections.

These early dose-ranging studies in the United Kingdom were followed by additional dose-ranging studies in Thailand. This is a report of those dose-ranging studies performed with atovaquone given alone, or in combination with other antimalarial drugs, during 1990–1993 in 317 Thai patients with acute malaria; all except one cohort of patients had falciparum malaria.

PATIENTS AND METHODS

Subjects were recruited for the study from patients who were admitted to the Bangkok Hospital for Tropical Diseases, Thailand, between October 1990 and June 1993 with acute, uncomplicated malaria. The studies were approved by the Ethical Committee of Mahidol University. Except for cohorts 8 and 9, male patients with parasite counts between 1,000 and 200,000/μl who were 16–65 years of age, had no underlying diseases, and who granted their informed consent were accepted into the study. Because the patients in cohorts 8 and 9 were treated with proguanil alone, patients with parasite counts less than 1,000/μl were accepted into these two cohorts. Those patients who had received incomplete antimalarial treatment prior to admission and whose symptoms had subsided were carefully observed until their parasitemia began to increase, and they developed acute manifestations of the disease, e.g. fever.

Patients with *P. falciparum* malaria were considered to have multidrug-resistant parasites based on previous studies in this same hospital.[5,6] Seventeen of the patients had previously been treated in the hospital for malaria with another antimalarial and had had recrudescences prior to discharge. These antimalarials included mefloquine, halofantrine, quinine, tetracycline, artemether, and artesunate. Patients with mixed infections, persistent diarrhea or vomiting, or with intercurrent febrile infections were excluded. All study subjects agreed to remain in hospital during a 28-day follow-up period, or in Bangkok where malaria transmission does not

TABLE 1
Drug regimens and therapeutic responses for patients with falciparum malaria*

Cohort	No.	Atovaquone dose	Companion drug	Dose regimen	PCT (hr)	FCT (hr)	Cure rate (%)
1	25	750 mg q8hr × 4 doses	None	None	64	59	72
2	23	750 mg q8hr × 21 doses	None	None	60	48	61
3	25	750 mg q8hr × 4 doses	Tetracycline	250 mg qid × 7 days	68	31	100
4	26	750 mg q8hr × 4 doses	Proguanil	250 mg qd × 7 days	61	43	96
5	30	500 mg bid × 3 days	Proguanil	200 mg bid × 3 days	60	46	93
6	22	500 mg bid × 3 days	Doxycycline	100 mg bid × 3 days	65	52	91
7	24	500 mg bid × 3 doses	Proguanil	200 mg bid × 3 doses	64	64	83
8	4	None	Proguanil	200 mg bid × 3 days	†	†	0
9	13	None	Proguanil	500 mg bid × 3 days	124	69	8
10	24	1,000 mg qd × 3 days	Pyrimethamine	25 mg qd × 3 days	64	43	75
11	24	1,000 mg qd × 3 days	Proguanil	400 mg qd × 3 days	65	61	100
12	24	500 mg bid × 5 days	Proguanil	200 mg bid × 5 days	71	85	100
13	13	500 mg qd × 3 days	Pyrimethamine	25 mg qd × 3 days	63	59	77

* PCT = parasite clearance time; FCT = fever clearance time; q8hr = every 8 hr; qid = four times a day; bid = twice a day; qd = every day.
† Parasites and fever not cleared.

occur. Two hundred ninety-six patients were followed for 28 days, or until their malaria recurred.

The therapeutic regimen used for treatment evolved during the three years of study in a series of patient cohorts, usually involving approximately 25 patients each (Table 1). The patients in all cohorts except cohort number 14 had acute falciparum malaria; the patients in cohort 14 had acute vivax malaria. The patients in cohort 14 were given the same therapeutic regimen as the patients in cohort 11.

All antimalarial drugs were administered under supervision. Since previous pharmacokinetic studies in volunteers had shown ingestion of food increased the bioavailability of atovaquone, the drugs were given 45 min after ingestion of liquid nourishment, usually consisting of Ovaltine® (Formost Friesland PCL, Bangkok, Thailand), soya milk, or fruit drink.[2]

Patients were monitored closely during the acute stage of their illness; pulse and body temperature were measured every 4 hr. Antipyretics were administered for temperatures greater than 39°C. Adequate fluid intake was maintained by oral fluids when tolerated, or by intravenous fluids as necessary.

Thick and thin blood films were prepared every 6 hr for determination of parasite counts until three consecutive films were negative. Thereafter, blood films were prepared daily. The films were stained with Giemsa and parasite counts were determined by counting the number of asexual parasites per 200 white blood cells or 1,000 red blood cells and expressing the results in parasites per microliter. Blood films were considered negative after the examination of 200 oil-immersion fields showed no asexual parasites.

Blood was also obtained prior to treatment for in vitro culture and assessment of drug sensitivity. The specimens were collected in heparin and preserved in liquid nitrogen.[7] In patients who experienced recrudescences, a second blood specimen was obtained before treatment with another drug and similarly preserved in liquid nitrogen. The paired specimens were subsequently cultured for concurrent determination of drug sensitivity to atovaquone and other antimalarials.[8] The results were expressed as the concentration of drug required for 50% inhibition of parasite growth (IC_{50}).

For patients with *P. falciparum* malaria, the response to treatment was adapted from the World Health Organization classification system.[9] A sensitive response (S) indicated parasite clearance within seven days without recrudescence during the 28-day follow-up period. Resistant responses were subdivided into R1 responses (parasite clearance within seven days, followed by recrudescence within 28 days) and R2-3 responses (failure to clear parasites during seven days). Those patients not followed for at least 28 days were excluded from analysis of efficacy. Cure rates were expressed as the percentage of S responses to the total number of evaluable patients in each cohort.

For patients with *P. vivax* malaria (cohort 14), the response to treatment was determined after only 14 days. Recurrent parasitemia between days 14 and 28 was recorded, but recrudescence could not be distinguished from relapse.

The rates of parasite clearance from the peripheral blood and resolution of fever were considered corroborative evidence of efficacy. Parasite clearance times were calculated from initiation of antimalarial treatment until the first time that peripheral blood films were negative for asexual parasites. Fever clearance times were similarly calculated from initiation of treatment until the body temperature had decreased to 37.5°C, and remained less than 37.5°C for at least 24 hr.

The safety of the various regimens was assessed by serial clinical observations, blood tests, and urinalyses. The patients were examined daily and questioned regarding any symptoms. The symptoms were recorded on a standardized checklist; symptoms not on the checklist were also recorded. Blood specimens were obtained prior to treatment, and on days 3, 7, 14, and 28 after initiation of treatment for routine hematology and clinical chemistry tests. Red blood cell glucose-6-phosphate dehydrogenase (G6PD) was also determined in all patients. Urinalyses were performed prior to treatment and on day 7. Most patients also had three stool examinations for parasites and a routine chest radiograph. Thirty-nine patients in cohorts 10 and 11 who were admitted during the daytime also had electrocardiograms performed prior to and 4 hr after all three doses of atovaquone.

Adverse experiences were defined as any clinical finding that first occurred, or increased in severity, during treatment or within seven days after completion of treatment. In each such instance, the principal investigator also recorded his judgment regarding the probability of the adverse experience being related to the treatment.

TABLE 2
Principal signs and symptoms before and after treatment

Symptom	Prior to treatment (%)	Following treatment (%)
Weakness	96.5	1.6
Headache	94.6	2.8
Fever	92.1	0.0
Myalgia	80.4	2.5
Anorexia	78.2	1.6
Nausea	77.0	1.3
Chills or rigors	70.7	0.9
Dizziness	70.0	2.2
Vomiting	48.9	2.2
Abdominal pain	19.9	5.7
Diarrhea	9.8	4.7
Insomnia	8.8	2.2
Palpitations	8.5	0.6
Pruritus	4.1	0.6
Dry mouth	2.2	0.0
Tinnitus	0.6	0.3
Hematochesia	0.0	0.6
Epistaxis	0.0	0.6
Rash	0.0	0.3
Arthralgia	0.0	0.3

RESULTS

Three hundred seventeen adult male patients participated in the studies. Thirty-nine, or 12.4%, had red blood cell G6PD deficiency. Two hundred ninety-two patients had acute falciparum malaria (mean age 25.7 years, mean weight 52.5 kg, and geometric mean initial parasite count 13,800/μl) and 25 patients had acute vivax malaria (mean age 21.5 years, mean weight 48.1 kg, and geometric mean initial parasite count 17,141/μl). Eighteen patients withdrew from the study for personal reasons unrelated to the drug treatment or side-effects (12 with falciparum malaria and six with vivax malaria). Three additional patients were re-entered into the study after having suffered recrudescences and were re-treated. These three patients had a poor response to re-treatment and were evaluated separately. The remaining 296 patients were followed for 28 days and were considered evaluable; their data were included in the calculations of drug efficacy. The therapeutic results for patients with falciparum malaria are summarized in Table 1.

These data show that atovaquone given alone (cohorts 1 and 2) cured only about two-thirds of patients, even when relatively large doses were administered for seven days. When either tetracycline or proguanil was given concurrently for seven days (cohorts 3 and 4), cure rates were greater than 95%. Efficacy remained high when the duration of treatment was shortened to five days (cohort 12) or three days (cohort 5-6) and doxycycline was substituted for tetracycline, but decreased to 83% when treatment was shortened to one day (cohort 7). Proguanil alone, even in doses of 1 g a day, was remarkably ineffective (cohorts 8 and 9). Pyrimethamine administered with atovaquone (cohorts 10 and 13) contributed only marginal improvement in cure rates over atovaquone alone. The most effective regimen administered during the shortest interval was atovaquone combined with proguanil given in single large daily doses for three days (cohort 11).

All 19 evaluable patients in cohort 14 with vivax malaria were clinically cured. Fourteen of the 19, however, had recurrent vivax parasitemia between 16 and 26 days after completion of treatment. These recurrent parasitemias were considered to be relapses from persistent exoerythrocytic infection, but recrudescences of erythrocytic infections cannot be excluded.

TABLE 3
Clinically significant laboratory abnormalities after treatment*

Test	Criteria	Number (%) developing the abnormality	Number abnormal at 28 days
Hematocrit	<25%	25 (7.8)	1
Hemoglobin	<7.5 g/dL	11 (3.5)	1
Red blood cell count	<3.0/pL	19 (6.0)	1
White blood cell count	<3,000/μl	5 (1.6)	0
Neutrophil count	<1,000/μl	17 (5.4)	1
Eosinophil count	>1,000/μl	157 (49.5)	112
Prothrombin time	>125% of control	5 (1.6)	0
PTT	>50 sec	7 (2.2)	0
BUN	>25 mg/dL	0 (0)	0
Creatinine	>2.0 mg/dL	0 (0)	0
Uric acid	>9.0 mg/dL	3 (0.9)	0
Total protein	<5.0 g/dL	1 (0.3)	0
Albumin	<3.0 g/dL	16 (95.0)	0
Bilirubin	>2.0 mg/dL	10 (3.2)	2
ALAT	>100 U/L	37 (11.7)	7
ASAT	>100 U/L	20 (6.3)	1
Sodium	<130 meq/L	1 (0.3)	0
Potassium	<3 or >6 meq/L	3 (0.9)	2
Chloride	<90 meq/L	1 (0.3)	1
Calcium	<8.0 mg/dL	17 (5.4)	1
Phosphorus	<2 or >6 mg/dL	3 (0.9)	0

* PTT = partial thromboplastin time; BUN = blood urea nitrogen; ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase.

Seventy-six of the 277 patients with falciparum malaria in cohorts 1–13 who completed their follow-up developed delayed primary attacks of vivax malaria. These patients were treated with small (450 mg) doses of chloroquine to eliminate erythrocytic forms of *P. vivax* until their follow-up period for *P. falciparum* was completed. At this time, they were given a full course of primaquine for elimination of exoerythrocytic forms.

The principal signs and symptoms occurring prior to treatment, and those appearing or increasing in severity within seven days after treatment are shown in Table 2. All the signs and symptoms observed are commonly reported in patients with acute malaria. In no cases were the reported symptoms attributed to the treatment regimen by the principal investigator.

More than 26,000 hematology and clinical chemistry tests were performed on the 317 subjects. The results of these tests reflected those changes commonly associated with acute malaria infections. Examination of means and/or medians of these data showed no unexpected or unusual trends. To examine individual data for possible drug related changes, the results of the laboratory studies were examined for patients who developed clinically significant results following treatment for malaria. The criteria used and the number of patients with abnormal results are shown in Table 3.

Although the development of these laboratory abnormalities occurred after initiation of treatment, they are commonly seen in evolving malaria infections. Seven of the 25 patients who had significant decreases in their hematocrit were

deficient in red blood cell G6PD. All except one, however, were anemic prior to drug treatment. Eosinophilia was common in this population. Approximately 84% of the patients had one or more enteric parasites. The most common were hookworm (57%), whipworm (41%), ascaris (30%), strongyloides (24%), and opisthorchis (17%). Treatment for intestinal parasitism was delayed until follow-up of their malaria infection was complete at 28 days. More than 10% of patients developed significant elevation of alanine aminotransferase levels. Although these changes are seen in evolving malaria infections, seven patients had persistent elevations at 28 days. Five of these seven patients had hepatitis B surface antigenemia. Although the elevated enzyme levels were attributed to concomitant hepatitis in these patients, the possibility of drug-related toxicity could not be excluded.

The electrocardiograms taken before and 4 hr after each dose in 39 patients in cohorts 10 and 11 showed no significant changes in PR or QTc intervals.

A total of 53 patients from cohorts 1–13 had recrudescent (or persistent) parasitemia after treatment. Paired *P. falciparum* isolates from 15 of these were cultured to assess parasite sensitivity to atovaquone and other antimalarial drugs (Kyle DE, unpublished data). The admission isolates from this group were uniformly susceptible to atovaquone (mean IC_{50} = 3.3 ng/ml, n = 12), but all were resistant to chloroquine and one or more other antimalarial drugs. Recrudescent isolates from patients treated with atovaquone alone showed marked resistance (IC_{50} = 4,947.1 ng/ml) although the development of resistance to atovaquone did not correlate with changes in susceptibility to any other antimalarial drugs. Remarkably, recrudescent isolates from patients treated with atovaquone plus proguanil showed only a minor decrease in susceptibility (IC_{50} = 3.8 ng/ml, n = 3) when compared with admission isolates from the same patients (IC_{50} = 1.2 ng/ml).

DISCUSSION

The continuing decrease in the susceptibility of *P. falciparum* to existing therapies in Thailand underlines the urgency for new drugs.[5,6]

Interest in the hydroxynaphthoquinones as antimalarial agents stems from the early 1940s following the observation that hydrolapachol was active against *P. lophurae* in an avian model.[1] This finding led to the synthesis of a number of hydrolapachol analogues in the United States during the World War II Antimalarial Drug Development Program.[10] This effort resulted in the synthesis of lapinone that was developed and tested in subjects with *P. vivax* malaria.[11] Although effective, the drug required parenteral administration for maximum activity and interest in the series decreased.

The advent of chloroquine resistance in the 1960s highlighted the need for additional antimalarial drugs and interest in the hydroxynaphthoquinones was revitalized. Studies by the U.S. Army identified menoctone as the most promising compound in the series based on results from the newly introduced mammalian model using *P. berghei* in mice. However, clinical studies of menoctone in volunteers infected with *P. falciparum* showed no activity when the drug was administered orally in doses of 500 mg/day for three days.[12] This disappointing response was attributed to poor bioavailability and again interest in the series lapsed.

During the 1970s, the hydroxynaphthoquinones were reevaluated by The Wellcome Research Laboratories in the United Kingdom where new compounds in the series were synthesized based on test results of *P. falciparum* in vitro. The mechanism of action of active drugs in the series was found to be inhibition of electron transport and hence dihydrooratate dehydrogenase.[13] The initial compound selected for development was 58C80. Human tolerance studies with 58C80 showed that this drug was rapidly metabolized to a hydroxylated derivative producing red urine. This problem of rapid metabolism was subsequently overcome with the development of an analogue, 566C80 or atovaquone. This analogue had a chloro-phenyl group instead of a tertiary butyl group in the side chain of the hydroxynaphthoquinone nucleus to block hydroxylation. Thus, atovaquone was selected for development based on high intrinsic activity, a novel mode of action through inhibition of electron transport, and metabolic stability. Human tolerance studies showed the drug was well tolerated and had an elimination half-life of approximately 70 hr.[2]

The early clinical studies of atovaquone for treatment of *P. falciparum* malaria were undertaken in the United Kingdom in a small number of patients who had acquired their infections abroad (Chiodini PL, unpublished data). Although consistently effective in clearing parasitemia following administration of a single dose of 500 mg, the majority of patients subsequently suffered recrudescences. Cure rates did not correlate with the source of infection, initial parasitemia, or maximum plasma concentrations of atovaquone. It was concluded that the dose and duration of treatment had been insufficient to effect consistent radical cures.

Based on this evidence, additional dose-ranging studies were performed in Thailand in patients with multidrug-resistant falciparum malaria. The dose of atovaquone was increased from single doses of 500 mg to multiple doses of 750 mg three times a day for 1–7 days. In common with the response in the United Kingdom patients, the Thai patients receiving these doses had prompt clinical and parasitologic responses. These results, however, were compromised by an unacceptably high rate of recrudescence of approximately 33%. Two patients who were re-treated with atovaquone failed to exhibit a clinical or parasitologic response and required treatment with alternative therapy after 48 hr.

This poor response to re-treatment with atovaquone suggested that parasite resistance was the probable explanation for the recrudescences. This was supported by in vitro susceptibility studies on paired isolates, taken prior to treatment and at the time of recrudescence, which revealed a marked increase in the IC_{50} for atovaquone.

In an attempt to overcome this problem, data from earlier laboratory studies of atovaquone in combination with other antimalarial drugs were reviewed and additional in vitro studies against *P. falciparum* conducted.[14] These studies showed consistent evidence of potentiation between atovaquone and either tetracycline or proguanil against both atovaquone-sensitive and resistant-isolates. The results of clinical studies with both of the drug combinations were dramatic. Not only was there a prompt clinical response, but radical cure rates approached 100%.

Further dose-ranging studies were used to optimize the therapeutic regimens. Proguanil was ultimately selected for coadministration on the basis of its ability to be used in pregnancy and young children. Remarkably, the combination of atovaquone and proguanil was effective despite clinical evidence that the strains of malaria in Thailand were highly resistant to proguanil when administered alone.

These continuing dose-ranging studies showed the combination of atovaquone and proguanil was curative in more than 97% of patients treated with a variety of dose regimens. Moreover, there was only a marginal increase in the IC_{50} of atovaquone in the recrudescent parasites from the few patients who were not cured with the combination. An optimal regimen of 1000 mg of atovaquone and 400 mg proguanil, once a day for three days, produced a 100% cure rate in 24 patients. This regimen is currently the focus of evaluation in a worldwide phase III program of comparative studies.

Limited studies in patients with vivax malaria showed the combination of atovaquone and proguanil to be effective in eliminating erythrocytic stages of this parasite. The subsequent recurrence of parasitemia was attributed to persistent exoerythrocytic forms (hypnozoites) and indicates that the drug combination has no significant activity on this form of *P. vivax*. The occurrence of early relapses following treatment with atovaquone and proguanil is similar to the case when volunteers with sporozoite-induced Chesson vivax malaria relapsed following treatment with quinine.[15, 16]

Re-treatment of the patients who suffer recrudescences after treatment with atovaquone is probably not warranted because resistance develops so rapidly as to render the compund ineffective. One patient who vomited shortly after taking three of four doses of atovaquone and proguanil suffered a recrudescence and was re-treated with the same combination. Similar to the two patients who were re-treated with atovaquone alone, this patient also failed to respond, was withdrawn from the study, and was treated with artesunate and mefloquine.

Atovaquone, both alone and in combination with proguanil, was very well tolerated and resulted in the early resolution of clinical symptoms. Serial observations on a range of hematologic and biochemical parameters did not reveal any consistent abnormality and where abnormal values did occur, they were considered to have been manifestations of the malarial infection.

In conclusion, the combination of atovaquone and proguanil, administered in a three-day dosing regimen, was shown to be both safe and highly effective in the treatment of acute uncomplicated multidrug-resistant falciparum malaria.

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