MALARONE® (ATOVAQUONE AND PROGUANIL HYDROCHLORIDE): A REVIEW OF ITS CLINICAL DEVELOPMENT FOR TREATMENT OF MALARIA

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Abstract. The continuing spread of drug-resistant malaria emphasizes the need for new antimalarial drugs. Atovaquone is a broad-spectrum antiprotozoal drug with a novel mechanism of action, via inhibition of parasite mitochondrial electron transport, and a favorable safety profile. Early studies with atovaquone alone for treatment of malaria demonstrated good initial control of parasitemia but an unacceptable rate of recrudescent parasitemia. Parasites isolated during recrudescence after treatment with atovaquone alone were resistant to atovaquone in vitro. The combination of atovaquone and proguanil is synergistic in vitro, and clinical studies demonstrated enhanced efficacy of the combination compared to either drug alone for treatment of malaria. Malarone®, a fixed-dose combination of 250 mg of atovaquone and 100 mg of proguanil hydrochloride, is available in many countries for treatment of acute, uncomplicated malaria caused by Plasmodium falciparum. At the recommended dose (in adults, four tablets once a day for three days), the overall cure rate was >98% in more than 500 patients with falciparum malaria. In four randomized, controlled clinical trials, treatment with atovaquone and proguanil hydrochloride was significantly more effective than mefloquine (Thailand), amodiaquine (Gabon), chloroquine (Peru and the Philippines) or chloroquine plus pyrimethamine/sulfadoxine (Philippines). In clinical trials where the comparator drug was highly effective, treatment with atovaquone and proguanil hydrochloride was equally effective. Parasites isolated during recrudescence after treatment with the combination of atovaquone and proguanil were not resistant to atovaquone in vitro. The most commonly reported adverse events in clinical trials (abdominal pain, anorexia, nausea, vomiting, diarrhea and coughing) occurred with similar frequency in patients treated with a comparator drug. Malarone is a safe and effective new agent for treatment of malaria.

The continuing spread of drug-resistant malaria and new appreciation of side effects associated with many existing antimalarial agents have emphasized the need for new drugs for prevention and treatment of malaria. Malarone® (Glaxo Wellcome, Inc., Research Triangle Park, NC), a fixed-dose combination of atovaquone and proguanil hydrochloride, is the first new antimalarial therapy in more than 40 years originating from industry-sponsored research. This combination is significantly more effective than either component alone, is effective against strains that are resistant to a variety of other antimalarial drugs, and has a favorable safety profile. In the present paper, we review the results of clinical studies that have demonstrated the safety and efficacy of atovaquone and proguanil hydrochloride and supported its registration for treatment of malaria.

MATERIALS AND METHODS

We reviewed primary data from 12 clinical studies conducted between 1990 and 1996 that evaluated atovaquone, alone or in combination with other drugs, for treatment of malaria (Glaxo Wellcome, Inc., unpublished data).1-10 These studies enrolled 1,395 patients with Plasmodium falciparum malaria and 32 patients with non-falciparum malaria, including 278 females and 200 children ≤12 years of age. Four uncontrolled studies evaluated 397 patients. Two studies (115-003 in the United Kingdom,1 115-005 in Thailand,2 and 115-012 in Zambia [Glaxo Wellcome, Inc., unpublished data]) evaluated atovaquone alone for treatment of malaria in 92 adults. These studies demonstrated that treatment with atovaquone alone had an unacceptable failure rate. Study 115-005 also evaluated proguanil alone (18 patients) or atovaquone in combination with a variety of other antimalarial drugs (248 patients), and identified proguanil hydrochloride as the optimal drug to be combined with atovaquone and also determined the optimal treatment regimen for this combination.2 One uncontrolled study in Thailand (115-123) evaluated the optimal regimen of atovaquone and proguanil hydrochloride for treatment of malaria in 32 children.7

Eight randomized, controlled studies (Table 1) compared the optimal regimen of atovaquone and proguanil hydrochloride versus standard antimalarial treatment in 1,030 patients living in endemic countries (seven studies) or returning to France (one study). Seven of these studies were in adults and one (115-131) was in children.

All studies enrolled patients with acute, uncomplicated falciparum malaria, and two studies (115-005 and 115-134) also enrolled patients with non-falciparum malaria. All patients were symptomatic and almost all had fever. Initial parasite counts were generally between 1,000 and 200,000/μl, and geometric mean parasite counts were approximately 5,000–30,000/μl. Patients were excluded from enrolling if they were pregnant or breast feeding, had complicated malaria or a concomitant disease that could mask assessment of the therapeutic response, had severe diarrhea, or were

unable to tolerate oral therapy. In the event of vomiting within 1 hr of dosing, a repeat dose was administered.

Patients were monitored closely during the acute stage of their illness. Clinical examinations were generally performed at least once a day for the first seven days after starting treatment and thereafter on days 14, 21, and 28. Thick and thin blood films for determination of parasite counts were prepared every 6–12 hr until three films were negative, daily to day 7, and weekly to day 28. Blood films were stained with Giemsa stain and parasites were numbered by counting the number of asexual parasites per 200 white blood cells or 1,000 red blood cells, expressing results as parasites per microliter of blood. A thick blood film was considered negative only after an examination of 200 oil-immersion fields showed no parasites.

Adverse events were defined as signs and symptoms that first occurred following treatment, or within 7–10 days after completion of treatment, or were present prior to treatment but increased in intensity after treatment. Signs or symptoms present prior to treatment that disappeared temporarily but recurred within 7–10 days after treatment were also included as new events. A serious adverse event was defined as any event that was fatal, immediately life-threatening, permanently or significantly disabling, necessitated or prolonged hospitalization, or was a congenital anomaly, malignancy, or overdose. Treatment-limiting adverse events were defined as those that caused the subject to stop taking medication and withdraw from the study.

Hematology and clinical chemistry parameters were generally monitored before and on days 3, 7, 14, and 28 after starting treatment. Hematology parameters included hemocrit, hemoglobin, red blood cell count, platelet count, and white blood cell count and differential count. Clinical chemistry parameters included total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, creatinine, blood urea nitrogen (BUN), and albumin. Laboratory data were analyzed by comparing changes in the means for laboratory parameters at various times after treatment and the proportion of patients who developed a markedly abnormal result at any time following treatment. The definitions of markedly abnormal results were hemocrit < 25%, hemoglobin level < 7.5 g/dl, red blood cell count < 3 × 10^12/L, platelet count < 50 × 10^9/L, white blood cell count < 3 × 10^9/L, neutrophil count < 1 × 10^9/L, eosinophil count > 1 × 10^9/L, total bilirubin > 2 mg/dl, AST > 100 U/L, ALT > 100 U/L, glucose < 50 mg/dl, creatinine > 1.5 mg/dl, BUN > 25 mg/dl, and albumin < 3 g/dl.

The primary efficacy endpoint in all studies was the parasitologic cure rate. Drug treatment is considered curative for *Plasmodium falciparum* malaria if parasites are eliminated from peripheral blood within seven days of starting treatment with no recrudescence within a 28-day follow-up observation period. Parasitologic outcomes were classified as sensitive (S) or resistant (R1, R2, or R3) according to the standard World Health Organization classification. Cure rates were calculated by dividing the number of patients with a sensitive response by the total number who completed the observation period. In most studies the patients were followed in a convalescent facility where reinfection was unlikely. In two studies (115-131 in Kenya and 115-134 in Gabon), however, patients were followed as outpatients and reinfection during the 28-day follow-up period was possible. Secondary efficacy endpoints were the parasite clearance time (PCT; the time required for parasites to be eliminated from peripheral blood films) and fever clearance time (FCT; the time for an individual patient to become afebrile). Because the efficacy parameters are objective and not dependent on the investigator’s assessment, blinding of the investigator was not considered necessary and was not used.

Yates’ corrected chi-square analysis was used to compare cure rates and rates of adverse events and markedly abnormal laboratory results. The Mann-Whitney U test was used to compare fever and parasite clearance times.

The appropriate ethics committees/institutional review boards approved all studies, and informed consent was obtained from all adult participants and from parents or legal guardians of minors.

**RESULTS**

**Efficacy evaluations.** *Evaluation of atovaquone alone.* A total of 89 evaluable patients with falciparum malaria were treated with atovaquone alone (Table 2) (Glaxo Wellcome, Inc., unpublished data). A single 500 mg dose was able to

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### Table 1

Randomized, controlled clinical trials of atovaquone and proguanil hydrochloride for treatment of acute, uncomplicated *Plasmodium falciparum* malaria

<table>
<thead>
<tr>
<th>Study number</th>
<th>Country</th>
<th>Number of subjects</th>
<th>Age range (years)</th>
<th>Comparator treatments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>115-120</td>
<td>Zambia</td>
<td>163</td>
<td>14–54</td>
<td>Pyrimethamine, 75 mg and sulfadoxine, 1,500 mg</td>
<td>8</td>
</tr>
<tr>
<td>115-122</td>
<td>Thailand</td>
<td>182</td>
<td>15–63</td>
<td>Mefloquine, 1,250 mg over a 6-hr period</td>
<td>6</td>
</tr>
<tr>
<td>115-127</td>
<td>Brazil</td>
<td>175</td>
<td>18–60</td>
<td>Quinine, 600 mg 3 times a day for 7 days and tetracycline, 250 mg 4 times a day for 7 days</td>
<td>5</td>
</tr>
<tr>
<td>115–130</td>
<td>France</td>
<td>48</td>
<td>15–62</td>
<td>Halofantrine, 500 mg every 6 hr × 3; repeated after 7 days</td>
<td>*</td>
</tr>
<tr>
<td>115-131</td>
<td>Kenya</td>
<td>168</td>
<td>3–12</td>
<td>Halofantrine, 50 mg/kg every 6 hr × 3</td>
<td>9</td>
</tr>
<tr>
<td>115-134</td>
<td>Gabon</td>
<td>141</td>
<td>15–80</td>
<td>Amodiaquine, 1,500 mg (base) over a 48-hr period</td>
<td>3</td>
</tr>
<tr>
<td>115-135</td>
<td>Philippines</td>
<td>110</td>
<td>12–64</td>
<td>Chloroquine, 1,500 mg (base) over a 48-hr period, alone or with pyrimethamine, 75 mg and sulfadoxine, 1,500 mg</td>
<td>10</td>
</tr>
<tr>
<td>115-136</td>
<td>Peru</td>
<td>43</td>
<td>15–65</td>
<td>Chloroquine, 1,500 mg (base) over a 48-hr period or pyrimethamine, 75 mg and sulfadoxine, 1,500 mg</td>
<td>*</td>
</tr>
</tbody>
</table>

* Glaxo Wellcome, Inc., unpublished data.
eliminate parasites from blood films, but parasitemia recurred within 10–23 days in 75% of the patients. Increasing the dose to 750 mg every 8 hr for four doses decreased the recrudescence rate to about 30%, but further dose escalation to 750 mg every 8 hr for 21 doses was also associated with a recrudescence rate of about 30%. All of these failures had an R1 pattern of resistance (recrudescence after initial clearance of patent parasitemia). After treatment with high doses of atovaquone alone, parasites isolated during recrudescent parasitemia were highly resistant to atovaquone in vitro. The mean atovaquone concentration required to inhibit parasite growth by 50% (IC$_{50}$) was 3.3 ng/ml for parasites obtained before treatment and 4.947 ng/ml for parasites obtained during the recrudescence infection. Two patients treated with 750 mg of atovaquone every 8 hr for 21 doses were re-treated with the same dose of atovaquone alone, and both had an R2 response to re-treatment. These data suggest that the parasite population was predominantly sensitive before treatment but, during treatment with atovaquone alone, a minor population of atovaquone-resistant mutant parasites was induced or selected and was able to replicate and cause recrudescence infection. Atovaquone alone is thus not an acceptable antimalarial drug.

**Evaluation of proguanil hydrochloride alone.** Although resistance to proguanil hydrochloride had been demonstrated in previous decades, there were no contemporaneous data on the clinical response to proguanil hydrochloride alone. Thus, a small number of patients in study 115-005 with mild falciparum malaria were treated with proguanil hydrochloride alone. None of the four patients treated with six doses of 200 mg of proguanil hydrochloride given at 12-hour intervals and only one of 13 patients treated with 6 doses of 500 mg proguanil hydrochloride given at 12-hour intervals were cured. The overall cure rate was one of 17 (6%) and most patients had evidence of high level resistance (R2 in 13 and R3 in two). Proguanil hydrochloride as a single agent is clearly not effective for the treatment of acute falciparum malaria in Thailand.

**Evaluation of atovaquone and tetracycline, doxycycline, or pyrimethamine.** A total of 84 evaluable patients with falciparum malaria were treated with atovaquone plus either tetracycline, doxycycline, or pyrimethamine. Treatment with atovaquone plus tetracycline or doxycycline resulted in acceptable cure rates, but tetracyclines may produce undesirable side effects, including permanent staining and enamel hypoplasia of developing teeth, and they should not be used in children 0–8 years of age or during the last half of pregnancy. The cure rate with atovaquone plus pyrimethamine was approximately 75% and was judged to be inadequate for treatment of acute malaria. Thus, none of these combinations was considered to be a preferred antimalarial regimen.

**Evaluation of a range of doses of atovaquone and proguanil hydrochloride in adults.** A range of doses of atovaquone in combination with proguanil hydrochloride was evaluated in cohorts of approximately 25 patients each. The cure rate with three doses of 500 mg of atovaquone plus 200 mg of proguanil hydrochloride at 12-hr intervals (24-hr total duration of treatment) was 83%. When the duration of treatment was extended to three days at the same dose, or when the duration of treatment with proguanil was extended to seven days, the cure rate increased to 93–96%. Limited data indicated that parasites isolated at the time of recrudescent parasitemia were not resistant to atovaquone in vitro. For three paired isolates, the mean atovaquone IC$_{50}$ was 1.2 ng/ml for parasites obtained before treatment and 3.8 ng/ml for parasites obtained during the recrudescence infection. This suggests that recrudescent parasitemia may not be associated with the emergence of drug-resistant parasites.

When the duration of treatment with 500 mg of atovaquone and 200 mg of proguanil hydrochloride twice a day was extended to five days, or when the dose was increased to 1,000 mg of atovaquone and 400 mg of proguanil hydrochloride once a day for three days, the cure rate was 100%. Although the size of the cohorts in these dose ranging studies was too small to detect statistically significant differences among treatment groups, the high cure rate with 1,000 mg of atovaquone and 400 mg of proguanil hydrochloride once a day for three days was sufficiently encouraging to justify further studies.

**Evaluation of the optimal regimen of atovaquone and proguanil hydrochloride.** In four controlled studies in adults (115-122, 115-134, 115-135, and 115-136), atovaquone and proguanil hydrochloride was significantly more effective than a comparator drug (Table 3). In these studies, the cure rate with atovaquone and proguanil hydrochloride (98–100%) was significantly better than the cure rate with mefloquine (86%), amodiaquine (81%), chloroquine (8% or 30%), or chloroquine plus pyrimethamine/sulfadoxine (87.5%).

In three controlled studies in adults (115-120, 115-127, and 115-130), atovaquone and proguanil hydrochloride had an efficacy similar to a comparator drug. In these studies, the cure rate was 99–100% with atovaquone and proguanil hydrochloride, 99% with pyrimethamine/sulfadoxine, 100% with quinine and tetracycline, and 100% with halofantrine.

In children, the dose of atovaquone and proguanil hydrochloride was adjusted for body weight categories (¼ dose for 11–20 kg, ½ dose for > 20–30 kg, ¾ dose for > 30–40 kg, and a full dose for > 40 kg) and was also administered once a day for three days. In an uncontrolled study in children (115-123), atovaquone and proguanil hydrochloride had an efficacy of 100%. In a controlled study in children (115-131), the efficacy of atovaquone and proguanil hydrochloride (94%) was similar to halofantrine (90%).

The overall efficacy of the optimal regimen of atovaquone and proguanil hydrochloride in 521 evaluable patients was 98.7% (Table 3). The seven patients who were not cured had

<table>
<thead>
<tr>
<th>Study</th>
<th>Atovaquone dose</th>
<th>No. treated</th>
<th>Outcome*</th>
<th>Cure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>115-003</td>
<td>500 mg × 1</td>
<td>8</td>
<td>S</td>
<td>6</td>
</tr>
<tr>
<td>115-005</td>
<td>750 mg every 8 hr × 4</td>
<td>25</td>
<td>S</td>
<td>18</td>
</tr>
<tr>
<td>115-005</td>
<td>750 mg every 8 hr × 9</td>
<td>4</td>
<td>S</td>
<td>3</td>
</tr>
<tr>
<td>115-005</td>
<td>750 mg every 8 hr × 21</td>
<td>23</td>
<td>S</td>
<td>14</td>
</tr>
<tr>
<td>115-012</td>
<td>750 mg every 8 hr × 21</td>
<td>29</td>
<td>S</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>89</td>
<td>S</td>
<td>59</td>
</tr>
</tbody>
</table>

* S indicates a sensitive response (elimination of parasitemia with no recurrent parasitemia during follow-up for 28 days) and R indicates a resistant response.
an R1 pattern of resistance. Efficacy was similar regardless of age, gender, or race. The cure rate was 104 (95%) of 109 among children ≤ 12 years of age and 410 (99.5%) of 412 among adults and adolescents ≥ 13 years of age; 381 (99%) of 386 among males and 133 (99%) of 135 among females; and 240 (97%) of 247 among blacks, 183 (100%) of 183 among Orientals, 39 (100%) of 39 among whites, and 52 (100%) of 52 among persons of mixed race.

Analysis of non-evaluable patients. In the controlled clinical trials, 85 (8%) of 1,030 patients were not evaluable. None of the non-evaluable subjects were withdrawn from the study because they developed complicated malaria or worsening disease. The response to treatment was unknown for 39 patients because they left the hospital or did not return to complete their 28-day follow-up evaluations. Forty-six patients were withdrawn from the study for various reasons. The rate of non-evaluable patients in these studies was low and evenly distributed among treatment arms, and thus would not be expected to distort the analysis of results.

Analysis of patients who failed treatment with atovaquone and proguanil hydrochloride. In the uncontrolled studies, seven of 128 evaluable patients treated for an initial episode of malaria were not cured (Table 3). Parasitemia cleared in all seven patients within seven days after initial therapy but recurrent parasitemia developed 19–28 days after starting treatment. Six of the seven (five in study 115-131 and one in study 115-134) were observed in the hospital for seven days or less and then completed their follow-up evaluation as outpatients, where they were potentially exposed to the bites of infected mosquitoes. Because the prepatent period for falciparum malaria averages about 10 days and can be as short as seven days after the bite of an infected mosquito,12 it is possible that many of these patients had a new infection rather than a recrudescence infection.

Three children with recurrent parasitemia after initial treatment with the optimal regimen of atovaquone and proguanil hydrochloride in study 115-131 were re-treated with the same regimen. Two of the three children were cured after the second course of treatment, suggesting that they had been re-infected. The third had an R2 response, suggesting that recurrent parasitemia in this patient was due to recrudescence of resistant parasites.

Among patients treated with the optimal regimen of atovaquone and proguanil hydrochloride, seven of 521 evaluable patients treated for an initial episode of malaria were not cured (Table 3). Parasitemia cleared in all seven patients within seven days after initial therapy but recurrent parasitemia developed 19–28 days after starting treatment. Six of the seven (five in study 115-131 and one in study 115-134) were observed in the hospital for seven days or less and then completed their follow-up evaluation as outpatients, where they were potentially exposed to the bites of infected mosquitoes. Because the prepatent period for falciparum malaria averages about 10 days and can be as short as seven days after the bite of an infected mosquito,12 it is possible that many of these patients had a new infection rather than a recrudescence infection.

Relationship between gastrointestinal symptoms and efficacy. Vomiting or diarrhea before treatment and the use of metoclopramide to treat vomiting did not affect efficacy of atovaquone and proguanil hydrochloride. Efficacy was 99.5% in the 189 patients who had vomiting as a pretreatment symptom and 100% in the 60 patients who had diarrhea as a pretreatment symptom. The use of metoclopramide is associated with a decrease in plasma atovaquone concentrations (Glaxo Wellcome, Inc., unpublished data), but only
Summary of adverse events in adults with acute *Plasmodium falciparum* malaria treated with the recommended dose of atovaquone and proguanil hydrochloride or with a comparator antimalarial drug in randomized, controlled studies

<table>
<thead>
<tr>
<th>Table 5</th>
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<table>
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<tr>
<th>% of patients with common adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>115-120</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Adverse event</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>n = 82</td>
</tr>
<tr>
<td>Abdominal pain</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Diarrhea</td>
</tr>
<tr>
<td>Asthenia</td>
</tr>
<tr>
<td>At least one event</td>
</tr>
<tr>
<td>Total A + P</td>
</tr>
</tbody>
</table>

* A + P = atovaquone and proguanil hydrochloride; P/S = pyrimethamine and sulfadoxine; MFQ = mefloquine; TCN = tetracycline; HLF = halofantrine; ADQ = amodiaquine; CQ = chloroquine.

one of the 56 patients whose vomiting was treated with metoclopramide during these studies was not cured after treatment with atovaquone and proguanil hydrochloride (efficacy = 98%).

In controlled studies in adults, a repeat dose of study drug was given because of vomiting within 1 hr of dosing in 33 of 436 patients treated with atovaquone and proguanil hydrochloride and 10 of 432 patients treated with a comparator drug (five of 71 with amodiaquine, two of 69 with chloroquine, one of 23 with halofantrine, one of 91 with mefloquine, and one of 122 with pyrimethamine/sulfadoxine). Among evaluable patients treated with atovaquone and proguanil hydrochloride, the cure rate was 96% (25 of 26) among patients who required a repeat dose of study drug given because of vomiting and 99.7% (385 of 386) among those who did not. Among evaluable patients treated with atovaquone and proguanil hydrochloride, the cure rate was 83% (10 of 12) among patients who required a repeat dose of study drug given because of vomiting, cure was achieved in three of four patients treated with amodiaquine and each of the four patients treated with one of the other comparator drugs.

In children, a repeat dose of study drug was given because of vomiting within 1 hr of dosing in 13 of 116 patients treated with atovaquone and proguanil hydrochloride and one of 84 patients treated with halofantrine. Among evaluable children treated with atovaquone and proguanil hydrochloride, the cure rate was 83% (10 of 12) among patients who required a repeat dose of study drug given because of vomiting and 96% (65 of 68) among those who did not. The patient who was given a repeat dose of halofantrine because of vomiting had an R1 response.

Secondary efficacy parameters. In the eight controlled studies, parasite clearance times were broadly similar between the groups of patients treated with atovaquone and proguanil hydrochloride (range of mean PCT = 44–72 hr) or a comparator antimalarial drug (range of mean PCT = 33–74 hr). Fever clearance times were also similar between the groups of patients treated with atovaquone and proguanil hydrochloride (range of mean FCT = 24–61 hr) or a comparator antimalarial drug (range of mean FCT = 20–51 hr). Small differences in PCT and FCT were statistically significant in some studies but were not considered to be clinically important.

Evaluation of atovaquone and proguanil hydrochloride in non-*falciparum* malaria. Twenty-five patients in Thailand with malaria caused by *P. vivax* were treated with 1,000 mg of atovaquone and 400 mg of proguanil hydrochloride once a day for three days in study 115-005 (Table 4). Among 23 patients evaluated for at least seven days, parasitemia cleared within seven days in 21 patients and by day 14 in the other two. All 19 patients evaluated on day 14 had no parasitemia and were considered clinically cured. However, 13 of the 19 had recurrent parasitemia on days 19–28 after starting treatment. Because patients were not treated concurrently with a drug known to be effective against the liver stages of *P. vivax*, it is likely that these recurrent parasitemias represented relapse of persistent hepatic infection, although recrudescence of erythrocytic infection cannot be excluded. Seven patients in Gabon with malaria caused by *P. ovale* or *P. malariae* were treated with 1,000 mg of atovaquone and 400 mg of proguanil hydrochloride once a day for three days (Table 4). All six evaluable patients were cured (three with *P. malariae*, two with *P. ovale*, and one with a mixed *P. falciparum* and *P. ovale* infection). The seventh patient had clearance of *P. ovale* parasitemia within seven days but was lost to follow-up thereafter.

Safety evaluations. There were 602 patients (486 adults and 116 children) with malaria who received the recommended treatment dose of atovaquone and proguanil hydrochloride, 94 who received atovaquone alone, 113 who received atovaquone and proguanil hydrochloride at other than the recommended treatment dose, 86 who received atovaquone in combination with a drug other than proguanil hydrochloride, and 516 who received a comparator drug.

Adverse events. Among adults treated with atovaquone and proguanil hydrochloride in controlled studies, the most frequently reported events were abdominal pain, headache, vomiting, nausea, diarrhea, and asthenia (Table 5). Among children, the most frequently reported events were coughing, headache, vomiting, abdominal pain, and anorexia (Table 6). All of these events were reported frequently as pre-treatment signs and symptoms. Most of the adverse events were mild or moderate in intensity. Although there was considerable variability in adverse event reporting rates among the studies, the nature and frequency of adverse events were generally similar between the atovaquone and proguanil hydrochloride group and comparator group within individual studies.

One death was reported during the conduct of these stud-
ies in a patient treated with atovaquone alone. He was a 23-year-old Zambian man with a history of psychiatric treatment four years earlier. He responded promptly to treatment with atovaquone alone (750 mg every 8 hr for seven days) with elimination of malaria symptoms and clearance of parasitemia by day 4. On day 5 he had a brief episode of bizarre behavior and subsequently became restless, agitated, anxious, and delusional with suicidal intentions. He continued to receive atovaquone for two additional days and was transferred to a psychiatric ward on day 10 where he was treated with haloperidol, benzhexol, and diazepam. On day 13 he became drowsy and a few hours later suddenly started gasping for breath and died before resuscitative measures could be taken. There was no autopsy. The investigator’s assessment was that the sudden death was not related to treatment with atovaquone, but the adverse events on day 5 were possibly attributable to atovaquone. There were nine additional patients who had an adverse event that was considered by the investigator to be serious. Of the 10 patients experiencing serious adverse events, two had been treated with atovaquone alone, five with atovaquone and proguanil hydrochloride, and three with a comparator drug. Four reported central nervous system events, four reported nausea and/or vomiting, and one each reported anaphylactic reaction, congestive heart failure, hemolysis, dental abscess, and cytomegalovirus. Events for only two patients were attributed to study medication (anaphylactic reaction in one and central nervous system events prior to the sudden death described above).

In addition to the patient described above, a second Zambian patient treated with atovaquone alone had psychiatric symptoms. The patient admitted alcohol and marijuana use four days prior to study entry. His psychiatric symptoms resolved spontaneously while receiving atovaquone and the event was considered not attributable to treatment with atovaquone.

Two patients experienced seizures after treatment with atovaquone and proguanil hydrochloride. Both had a history of seizures and the investigators considered the events not attributable to treatment. A Kenyan child had generalized convulsions after two doses of atovaquone and proguanil hydrochloride. She was treated with diazepam and phenobarbital and continued to receive atovaquone and proguanil hydrochloride without additional difficulty. A Peruvian woman had seizures 15 hr after her third dose of atovaquone and proguanil hydrochloride. Her serum sodium concentration was 115 mEq/L and the seizures were attributed to hyponatremia of unknown cause.

One patient had an anaphylactic reaction attributed to treatment with atovaquone and proguanil hydrochloride. She vomited repeatedly after each dose, even when premedicated with metoclopramide, and developed an urticarial skin reaction over the abdomen and hypotension (blood pressure = 70/45 mm of Hg). These symptoms subsided each afternoon but returned after the next dose on each day of treatment.

Two patients reported nausea and/or vomiting that was considered serious, one each after treatment with mefloquine or atovaquone and proguanil hydrochloride. Vomiting was present before entering the study and neither event was attributed to the study drug.

Three other patients had serious adverse events not attributed to the study drug: a serious dental abscess and cytomegalovirus infection in a patient treated with atovaquone and proguanil hydrochloride and congestive heart failure or blackwater fever in two patients treated with quinine and tetracycline.

Eight subjects had a treatment-limiting adverse event (four serious and eight non-serious events). Two patients treated with atovaquone and proguanil hydrochloride had a serious adverse event (anaphylaxis or vomiting) that was treatment-limiting. Two patients treated with a comparator drug had a serious adverse event (vomiting with mefloquine, blackwater fever with quinine) that was treatment-limiting. Four subjects had non-serious vomiting that was treatment-limiting (three treated with atovaquone and proguanil hydrochloride and one treated with atovaquone alone).

**Laboratory parameters.** Analysis of mean data showed that patients with acute malaria infections had evidence of anemia, mild reductions in platelet counts, and mild abnormalities in test results of hepatic and renal function, all of which are common in evolving malaria infections. White blood cell differential counts usually showed no abnormality until patients began to recover. Many patients had underlying intestinal parasites and manifested eosinophilia. Laboratory abnormalities tended to return towards normal levels with treatment in all patient groups. Evaluation of laboratory parameters showed no superimposed adverse trends that could be attributed to treatment with atovaquone, proguanil hydrochloride, or atovaquone in combination with proguanil hydrochloride or other drugs. In the controlled trials, the evolution of hematology and clinical chemistry parameters during and after treatment were similar in patients treated with atovaquone and proguanil hydrochloride or a comparator drug (Figure 1).

In study 115-122 in Thailand, markedly elevated levels of ALT or AST (> 100 U/L) occurred in 21% of the patients after treatment with atovaquone and proguanil hydrochloride and 9% of the patients after treatment with mefloquine (P < 0.05). Markedly elevated levels of ALT or AST also occurred commonly in the adults in study 115-005 in Thailand and in study 115-130 in France, but not in adult or pediatric patients in controlled clinical trials in other countries after treatment with atovaquone and proguanil hydrochloride or a comparator drug. Markedly elevated levels of bilirubin (> 2 mg/dl) also occurred disproportionately after treatment with...
Atovaquone is a hydroxynaphthoquinone with activity against Plasmodium spp., Pneumocystis carinii, and Toxoplasma gondii in vitro and in animal models. It has a novel mechanism of action, and favorable preclinical and clinical safety profile. Initial clinical studies demonstrated that atovaquone was effective at clearing patent parasitemia, but recrudescence infection (R1 resistance) developed in about 30% of the patients treated with atovaquone alone (Table 2). Recrudescence after treatment with atovaquone alone was associated with evidence of atovaquone-resistant parasites in vitro, and patients who were re-treated with atovaquone had clinical evidence of greater (R2) drug resistance. An extensive in vitro evaluation of drugs that might potentiate the activity of atovaquone was performed, and proguanil was identified as the most likely clinical partner for combination therapy. Groups of patients were then treated with atovaquone in combination with other antimalarial drugs, and proguanil hydrochloride, which was not effective as monotherapy, was best able to enhance the in vivo efficacy of atovaquone.

One patient in study 115-005 who received atovaquone and proguanil hydrochloride had hemoglobinuria and hemolysis that required blood transfusion. This patient was one of 71 who were deficient in red blood cell glucose-6-phosphate dehydrogenase and were treated with atovaquone and proguanil hydrochloride (52 patients) or atovaquone alone (19 patients). None of the other 70 patients had evidence of anemia out of proportion to their acute malaria infections.

DISCUSSION

Atovaquone is a hydroxynaphthoquinone with activity against Plasmodium spp., Pneumocystis carinii, and Toxoplasma gondii in vitro and in animal models. It has a novel mechanism of action by inhibiting the electron transport system at the level of cytochrome bc1 complex. In malaria parasites, there is an obligatory coupling of pyrimidine biosynthesis and electron transport, via ubiquinone/ubiquinol. The selective toxicity of atovaquone towards P. falciparum is achieved by virtue of the different sensitivities of mammalian and plasmodial electron transport systems to hydroxynaphthoquinones, and also by the fact that Plasmodium spp. are dependent on de novo pyrimidine biosynthesis while mammalian cells are able to salvage and recycle pyrimidines. The inability of malaria parasites to salvage preformed pyrimidines results in atovaquone blocking nucleic acid synthesis and thus replication. In vitro, atovaquone has an IC50 value against various P. falciparum strains of 0.7–4.3 nM, being consistently more potent than chloroquine, which has an IC50 value of 74–633 nM for the same strains.

Based on its potent in vitro activity against P. falciparum, efficacy in animal models of malaria, novel mechanism of action, and favorable preclinical and clinical safety profile, atovaquone was evaluated as a potential new antimalarial drug. Initial clinical studies demonstrated that atovaquone alone was effective at clearing patent parasitemia, but recrudescence infection (R1 resistance) developed in about 30% of the patients treated with atovaquone alone (Table 2). Recrudescence after treatment with atovaquone alone was associated with evidence of atovaquone-resistant parasites in vitro, and patients who were re-treated with atovaquone had clinical evidence of greater (R2) drug resistance. An extensive in vitro evaluation of drugs that might potentiate the activity of atovaquone was performed, and proguanil was identified as the most likely clinical partner for combination therapy. Groups of patients were then treated with atovaquone in combination with other antimalarial drugs, and proguanil hydrochloride, which was not effective as monotherapy, was best able to enhance the in vivo efficacy of atovaquone.

Atovaquone and proguanil hydrochloride were also conducted in Thailand. Atovaquone and proguanil hydrochloride was highly effective in all geographic areas tested, and was significantly more effective than mefloquine, amodiaquine, chloroquine, or the combination of chloroquine and pyrimethamine/sulfadoxine (Table 3). The studies in Africa were conducted in areas of holoendemic malaria; thus, most of the patients were semi-immune. Atovaquone and proguanil hydrochloride was highly effective in these patients, and also in patients in other countries who were generally non-immune. Atovaquone and proguanil hydrochloride has also been effective for treatment of multidrug-resistant malaria that could not be cured with other antimalarial drugs.

Atovaquone and proguanil both have favorable safety profiles. Atovaquone has been marketed since 1992 for the

**Figure 1.** Changes in laboratory parameters after treatment of acute Plasmodium falciparum malaria with atovaquone and proguanil hydrochloride (solid symbols) or a comparator drug (open symbols) in randomized, controlled studies. Values are the mean ± SEM. ALT = alanine aminotransferase; WBC = white blood cells.
The tablet formulation was approved initially at a dose of 750 mg three times a day for 21 days. The suspension formulation, which has oral bioavailability approximately twice that of the tablet formulation, was approved in 1995 at a dose of 750 mg twice a day for 21 days. Both the tablet and suspension formulations have been generally safe and well tolerated at these doses, with very low rates of serious adverse events. Daily doses of atovaquone for treatment of PCP are higher than the daily doses used for treatment of malaria. Overdoses of atovaquone as large as 31,500 mg have caused little or no symptomatology (Glaxo Wellcome, Inc., unpublished data). Proguanil hydrochloride has been used since the 1940s for treatment and prevention of malaria. Overdoses of proguanil hydrochloride as large as 15,000 mg have been followed by complete recovery, and doses as high as 700 mg twice a day have been taken for more than two weeks without serious toxicity. Preclinical and clinical studies with atovaquone and proguanil hydrochloride in combination have demonstrated no pharmacokinetic interactions and no additional toxicity compared with the use of either component alone.

The frequency and nature of adverse events reported in controlled clinical studies were generally similar in patients treated with atovaquone and proguanil hydrochloride or a comparator antimalarial drug (Tables 5 and 6). Most of the adverse events were present before starting treatment and were reported as pretreatment signs and symptoms. These results suggest that the adverse events are largely due to the disease rather than to the study drugs. Treatment-limiting adverse events occurred in less than 1% of the patients treated with atovaquone and proguanil hydrochloride, and serious adverse events attributable to treatment were rare. Hematology and clinical chemistry data support the view that atovaquone and proguanil hydrochloride is well tolerated. Transient elevations in liver function test results were reversible and not associated with untoward clinical events.

Vomiting is a common symptom in patients with malaria, and vomiting shortly after administration of an oral drug may interfere with drug absorption. Vomiting within 1 hr of dosing occurred in 8% of the adults and 11% of the children treated with atovaquone and proguanil hydrochloride. Such patients were managed by re-administration of the dose, and less than 1% of the patients required withdrawal from the study and treatment with an alternative antimalarial drug. Although 37 of 40 evaluable patients who were given a repeat dose of atovaquone and proguanil hydrochloride were cured, it would seem prudent to monitor such patients more closely than patients who do not require repeat doses.

The combination of atovaquone and proguanil hydrochloride also has activity against the erythrocytic stages of *P. vivax*, *P. ovale*, and *P. malariae* (Table 4). Recurrent parasitemia after treatment of vivax malaria with atovaquone and proguanil hydrochloride suggests that the regimen used does not have activity against hypnozoites of *P. vivax*. In a previous study in Thailand, about one-third of patients treated for falciparum malaria, but without *P. vivax* parasitemia at the time of treatment, developed *P. vivax* parasitemia within a few weeks after plasma drug concentrations decreased to levels that do not inhibit blood-stage parasites. This implies relapse from dormant liver-stage parasites (hypnozoites) that were not susceptible to the antimalarial drugs used. Additional studies are in progress to evaluate sequential therapy of vivax malaria with atovaquone and proguanil hydrochloride for three days followed by primaquine, which is active against hypnozoites of *P. vivax*, to prevent relapse. Preliminary results indicate this treatment regimen will be highly successful (Looareesuwan S, unpublished data).

The mechanism of *P. falciparum* resistance to atovaquone has been investigated by DNA sequence analysis of pretreatment and recrudescent isolates obtained from patients in a treatment trial. Recrudescent isolates from five patients treated with atovaquone alone showed markedly decreased sensitivity to atovaquone, and each had a point mutation in the cytochrome b gene, resulting in a predicted amino acid substitution (Wilson C, unpublished data). The overall incidence of treatment failure in patients treated with atovaquone alone was approximately 30%, and excluding the few patients who were re-treated during their recrudescent parasitemia, all such failures were of the R1 resistance pattern (recrudescence after initial clearance of patent parasitemia). Recrudescence is expected to occur whenever the initial number of parasites is greater than or equal to the inverse of the frequency of resistance. Assuming an average patient has a blood volume of six liters and an initial parasitemia between 1,000 and 200,000/µl, the total body parasite burden at the start of treatment is between 6 × 10⁶ and 1.2 × 10¹³. Thus, the effective rate at which the atovaquone resistance mutation occurs is approximately 10⁻¹⁰ to 10⁻¹². This is consistent with results of studies in which atovaquone-resistant *P. falciparum* was selected by drug exposure *in vitro*. The rate at which atovaquone-resistant parasites are detected is approximately 10⁻⁹ with exposure to 10⁻⁷ M atovaquone (0.04 µg/ml) and lower (undetectable in the *in vitro* system) with exposure to higher concentrations of atovaquone. During treatment with atovaquone and proguanil hydrochloride, maximum plasma concentrations of atovaquone are approximately 5 µg/ml after the third dose and approximately 1 µg/ml seven days after starting treatment. The enhanced clinical efficacy of atovaquone in combination with proguanil hydrochloride may be due to synergy between these two drugs or to a reduction in the rate at which parasites resistant to both drugs develop.

The studies summarized above provide substantial evidence that atovaquone and proguanil hydrochloride is safe and effective for treatment of malaria caused by *P. falciparum*, and limited data indicate that this combination is also active against the blood stages of *P. vivax*, *P. ovale*, and *P. malariae*. The combination is clearly more effective than either component alone and is significantly more effective than mefloquine, chloroquine, amodiaquine, or pyrimethamine/sulfadoxine in areas where parasites are resistant to these drugs. Because of its superior efficacy, novel mechanism of action, and activity against parasite strains resistant to other antimalarial drugs, the combination of atovaquone and proguanil hydrochloride is an important new alternative for treatment of malaria.

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