A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED FIELD TRIAL TO DETERMINE THE EFFICACY AND SAFETY OF MALARONE® (ATOVAQUONE/PROGUANIL) FOR THE PROPHYLAXIS OF MALARIA IN ZAMBIA

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Abstract. Malaria poses a major health risk to people who are exposed to infection in malaria-endemic areas. A randomized, double-blind, placebo-controlled study was conducted to determine the efficacy and safety of Malarone® (250 mg of atovaquone/100 mg of proguanil hydrochloride per tablet) for the chemoprophylaxis of Plasmodium falciparum malaria in Zambia. Adult volunteers received a three-day treatment course of Malarone to eliminate pre-existing parasitemia and were then immediately randomized to treatment with either one Malarone tablet daily (n = 136), or one placebo tablet daily (n = 138) for at least 10 weeks. Malaria blood smears were prepared on a weekly basis and a failure of chemoprophylaxis was defined as any subject who had a positive blood smear, or who withdrew from the study due to a treatment-related adverse event. The prophylaxis success rates in the Malarone and placebo groups were 98% and 63%, respectively (P < 0.001). The most commonly reported adverse events with at least a possible causal relationship to study medication were headache and abdominal pain, which occurred with a higher incidence in the placebo group. No subjects were withdrawn from the study due to a treatment-related adverse event. Thus, Malarone appears to have an excellent safety and efficacy profile for the chemoprophylaxis of P. falciparum infection.

Despite efforts to contain the disease, malaria remains one of the greatest causes of morbidity and mortality in the tropical and subtropical parts of the world. The World Health Organization estimates that about 300–500 million clinical cases of malaria occur annually and that about 1.5–2.7 million people die from the disease each year.3

Plasmodium falciparum is the most virulent parasite and is responsible for the high morbidity and mortality associated with the disease. This parasite is also the predominant species in sub-Saharan Africa, eastern Asia, Oceania, and the Amazon region and therefore poses a major risk not only to the people living in these areas but also to those traveling there. It is estimated that malaria is contracted by as many as 30,000 American and European travelers each year.4

Drugs or drug combinations that are currently prescribed for the prophylaxis of malaria include chloroquine and proguanil hydrochloride, mefloquine, doxycycline, and primaquine. Unfortunately, the parasite has become resistant to most of these drugs, notably in Southeast Asia, where daily doxycycline5 and possibly primaquine6 are the only reasonably effective drugs. However, both primaquine and doxycycline are associated with a high incidence of adverse effects and consequently there remains an urgent need for new, safe and effective, antimalarial chemoprophylactic drugs.4

Malarone® (Glaxo Wellcome, Inc., Research Triangle Park, NC) tablets comprise a fixed dose combination of two widely prescribed anti-infective drugs, namely, atovaquone and proguanil hydrochloride, in a ratio of 2:5:1. Atovaquone (Mepron®/Wellvone®, Glaxo Wellcome, Inc.) is marketed for the acute treatment of Pneumocystis carinii pneumonia in immunocompromised patients, while proguanil hydrochloride has been widely used since the 1940s as an antimalarial drug.

Atovaquone belongs to the hydroxynaphthoquinone class of compounds. The drug has been shown to have a novel mode of antimalarial action. In P. falciparum, it selectively inhibits mitochondrial electron transport,7 diminishes pyrimidine biosynthesis,8 and collapses mitochondrial membrane potential,9 ultimately preventing parasite replication.

Proguanil and its main active metabolite, cycloguanil, exert their antimalarial action through inhibition of plasmodial dihydrofolate reductase,10 leading to depletion of the pyrimidine nucleotide pool, thus blocking nucleic acid synthesis and ultimately cell replication.

Malarone has been developed for the treatment of P. falciparum infection. A series of controlled clinical trials conducted around the world, including areas of multi-drug resistance, have shown that the combination of atovaquone plus proguanil hydrochloride is both highly efficacious and safe in the treatment of acute, uncomplicated, P. falciparum malaria.11–14

Drugs that are effective for the treatment of malaria are also usually efficacious in the prevention of malaria. Results from initial field studies in both East and West Africa suggest that Malarone is indeed a highly efficacious product for the chemoprophylaxis of P. falciparum infection in both adults15 and children.16 The aim of this field study was to confirm the efficacy, safety and tolerance of Malarone for the chemoprophylaxis of P. falciparum malaria in adults at high risk of infection in a malaria-endemic area of Zambia.

PATIENTS AND METHODS

Study design and subject population. This was a double-blind, placebo-controlled, randomized, two-arm, parallel group trial. The study population included healthy male or female volunteers 18–65 years of age residing in a highly malarious area of Zambia. Subjects were excluded from the study if they were pregnant or unwilling to avoid pregnancy, lactating, would normally receive malaria prophylaxis during the high transmission season, slept under a bed net, had received drugs with antimalarial activity within the previous two weeks, had known hypersensitivity to atovaquone or proguanil, had clinically significant abnormal baseline hematologic or clinical chemistry laboratory values, had significant renal impairment, had undergone a splenectomy, showed laboratory evidence of hepatitis (three-fold increase above the normal upper limit in the serum alanine amino-
transferase level), or had clinically significant concomitant medical problems as determined by the investigators. All subjects gave informed consent and the protocol was reviewed and approved by the Institutional Review Board of the Tropical Diseases Research Center, Ndola, Zambia.

**Treatment assignment.** The study comprised three phases.

**Radical cure phase.** Following an initial screening visit, all eligible volunteers received a curative treatment regimen of four Malarone tablets (250 mg of atovaquone/100 mg of proguanil hydrochloride) administered once a day for three consecutive days to clear any pre-existing parasitemia prior to chemoprophylaxis.

**Prophylaxis phase.** Following the radical cure phase, subjects were immediately randomized to one of two suppressive prophylactic treatment regimens for at least 10 weeks (70 days), namely, one Malarone tablet a day, or one placebo tablet a day. Placebo consisted of an identical-appearing tablet composed primarily of lactose.

**Follow-up phase.** Following the prophylaxis phase of the study subjects entered a follow-up phase for up to four weeks.

Studies have shown that the ingestion of food increases the bioavailability of atovaquone and consequently all treatment regimens were administered within 45 min of a small meal. Medication was taken under direct supervision of trained field workers and compliance was further confirmed by a tablet count.

**Clinical assessment and efficacy endpoints.** The primary efficacy measure and endpoint for the study was the development of patent parasitemia on blood smear during chemoprophylaxis. Thick blood films were stained with Giemsa stain and the malaria parasite counts were determined by the number of asexual parasites per 200 white blood cells. A blood slide was not considered to be negative until an examination of 200 oil-immersion fields showed no parasites. The first confirmed instance of positive parasitemia with *Plasmodium* species occurring after the radical cure phase and during the daily administration of chemoprophylaxis was considered a failure of prophylaxis, and the subject was withdrawn and treated appropriately.

Because this was the primary study endpoint, each report of parasitemia that could be an indication of prophylaxis failure was confirmed as follows: a slide read as positive by one microscopist was reviewed by another microscopist. If they agreed that a given slide was positive, a second sample was quickly obtained from the volunteer for a second, confirmatory, thick film. If two microscopists agreed that the confirmatory thick film was positive, then that volunteer was classified as a prophylactic failure. The senior technologist present adjudicated disagreements among two microscopists. Volunteers whose confirmatory smears were negative within 24–72 hr continued to receive medication and were not counted as prophylaxis failures.

During the screening phase volunteers were assessed for their eligibility to enter the study and after signing an informed consent form the following was carried out: demography data were collected, a brief physical examination was performed, vital signs were measured, and a blood sample was taken for baseline hematology (hemoglobin, lymphocytes, hematocrit, platelets, total white blood cells) and clinical chemistry (alanine aminotransferase, albumin, alkaline phosphatase, creatinine, serum γ-glutamyltransferase, glucose, potassium, sodium, total bilirubin, urea). In the case of females a pregnancy test was performed. Volunteers who satisfied the eligibility criteria began the three-day curative treatment phase, followed immediately by chemosuppression for 10 weeks. Body temperature and adverse events since the last visit were recorded on a weekly basis. Thick blood films were taken at weekly visits and at any time that malaria was suspected. Blood samples were taken for routine hematology, clinical chemistry, plasma drug level determination, and pregnancy testing during week 5 and week 10 of chemosuppression. During the follow-up phase, volunteers were seen weekly for up to four weeks and the following were assessed: temperature, thick blood films and adverse events.

**Safety analysis.** Adverse events were listed together with the event’s intensity, investigator-attributed causality, onset, and cessation. All safety data were listed highlighting clinical laboratory values outside the normal range.

**Statistical analysis.** The malaria attack rate from historical data in the greater study area was estimated to be 30% or higher in the placebo treatment group and 1.5% or lower in the Malarone treatment group. A study with 134 volunteers in each treatment group would have a power of 80% to detect a 17% difference in efficacy rate at the 5% significance level (two-sided). To allow for dropouts, at least 150 volunteers were recruited into each treatment group.

A per-protocol analysis of the study results was performed because this is more clinically relevant. The per-protocol population was defined as those subjects who were randomized to receive either placebo or Malarone during chemosuppression, who received at least one dose of treatment during chemosuppression, who were protocol compliant with receiving medication, who did not receive concomitant medication that could influence the evaluation of efficacy, who had both a negative baseline smear following the three-day curative treatment regimen and were present at, or failed before week 10. Subjects who withdrew for any reason other than parasitemia or a treatment-related adverse event were excluded from the per-protocol population. The safety population included all subjects who received at least one chemoprophylactic dose. The prophylactic success proportions for the two groups (placebo and Malarone) were compared by considering the 2 × 2 frequency table and performing a Fisher’s exact test. Additionally, a point estimate and exact confidence interval was also calculated for the percent efficacy in which percent efficacy = 100 × (1 − [failure rate in the Malarone group/failure rate in the placebo group]). A failure of chemoprophylaxis included subjects who either had a positive blood smear (any *Plasmodium* species), or withdrew due to a treatment-related adverse event. A difference was considered statistically significant if \( P \) was < 0.05. No hypothesis testing was carried out on safety or demographic variables. Hodges-Lehmann estimates for the median treatment differences and 95% confidence intervals were calculated for hematology and clinical chemistry data.

**RESULTS**

Two hundred ninety-nine volunteers were screened and enrolled into the three-day curative treatment phase of the trial. A total of 274 (92%) volunteers completed the three-
day curative phase and were randomized to treatment with either one Malarone tablet (n = 136), or placebo (n = 138), of whom 213 (78%) were included in the per-protocol efficacy analysis.

Of the 25 subjects who did not complete the three-day curative phase of the trial, the principal reasons were protocol violation (n = 9), lost to follow-up (n = 8), consent withdrawn (n = 6), or other (n = 2). Of the 61 unevaluable subjects in the chemoprophylaxis phase, 30 (14 taking placebo and 16 taking Malarone) were lost to follow-up, 22 were protocol violators (8 taking placebo and 14 taking Malarone), and nine withdrew their consent (5 taking placebo and 4 taking Malarone). No subjects were withdrawn due to an adverse event.

The two chemoprophylactic groups at screening were similar with respect to demographic characteristics (Table 1). Twenty-one (7%) of the subjects had a positive malaria smear prior to curative treatment, 20 of whom had negative smears at the first visit (week 0) following the three-day curative treatment course of Malarone. One subject was lost to follow-up before week 0.

**Prophylactic efficacy.** A total of 41 subjects treated with placebo and two subjects treated with Malarone developed parasitemia. The prophylaxis success rates in the Malarone and placebo treatment groups were thus 98% and 63%, respectively (Table 2). The difference in success rates between placebo and Malarone was highly significant (P < 0.001).

The Malarone efficacy rate was 95% (95% confidence interval = 79–100%).

During the four-week follow-up phase of the study, parasitemia on blood smear was detected in six (9%) of 70 evaluable subjects in the placebo group and one (1%) of 100 evaluable subjects in the Malarone group. The single case in the Malarone group was detected at the last scheduled visit (week 14).

**Plasma drug levels.** The summary statistics of the average steady-state plasma trough concentrations (C\text{ss}) for atovaquone, proguanil, and cycloguanil as well as the ratio of proguanil to cycloguanil using pooled trough sampling times are shown in Table 3. Two subjects in the Malarone treatment arm developed parasitemia at week 6. At week 5, one of these subjects had a C\text{ss} for atovaquone of 3.39 µg/ml, which exceeded the mean C\text{ss} for the population: however, his proguanil and cycloguanil plasma concentrations were below the limit of quantification (5 ng/ml). The other subject had C\text{ss} values for atovaquone, proguanil, and cycloguanil of 0.74 µg/ml, 10.8 ng/ml, and 6.0 ng/ml, respectively, which were below the mean C\text{ss} levels.

**Safety.** During chemoprophylaxis, the most commonly reported adverse events irrespective of causality were headache, fever, and abdominal pain. The most commonly reported adverse events with at least a possible causal relationship to study medication were headache (9% in the placebo group and 4% in the Malarone group) and abdominal pain (5% in the placebo group and 3% in the Malarone group) (Table 4). Overall, the incidence of adverse events was higher in the placebo group.

No subjects were withdrawn from the study due to a treatment-related adverse event. Two subjects experienced serious adverse events (hospitalization) during the study. Both subjects were in the placebo group and their adverse events (lower respiratory tract infections) were considered unlikely to be related to study medication. Both of the serious adverse events resolved during follow-up evaluations. No treatment-emergent effects were evident for any of the hematology or clinical chemistry parameters measured.
The rationale for the evaluation of Malarone for the chemoprophylaxis of malaria was based on the synergistic activity of atovaquone and proguanil hydrochloride shown in vitro and in the treatment studies, the good safety profile of each component established in both short and longer term use, and the tolerance and safety of the combination established with the three-day therapeutic treatment regimen.

Malaria prophylaxis studies can be conducted either in subjects living in endemic areas or, alternatively, in travelers who are visiting endemic areas. However, it is extremely difficult to obtain meaningful results from studies in travelers due to variability related to the seasonality of malaria, destination of travelers once in an endemic country, exposure time, number of bites per day (human-biting rate), infectivity of mosquitoes (sporozoite rate), strain differences, preventative measures (clothing, insect repellents, bed nets), compliance, and concomitant medications.

A more reliable way of conducting malaria prophylaxis studies is to undertake these trials in subjects living in endemic areas. These studies have advantages in that field trials can be conducted during periods of peak malaria transmission to ensure that study subjects are maximally exposed to infection; the majority of subjects living in endemic areas do not have access to, or would not take malaria prophylactic drugs and consequently it is possible to conduct placebo-controlled trials in these volunteers; patient compliance can be more closely monitored in a community-based field study; and efficacy, safety, and tolerance can be more closely monitored. Although subjects living in endemic areas may have a partially protective immunity to the symptoms of malaria, this does not protect them from patent infection, thus the primary efficacy measure and endpoint for this study was the development of patent parasitemia on blood smear.

The results of this large field trial in adult volunteers indicate that 98% of the subjects receiving chemoprophylaxis with one Malarone tablet taken once a day for 10 weeks did not develop *P. falciparum* infection. The overall efficacy of Malarone relative to placebo was 95%. These results support the data from a recent study in Gabon, where Malarone was shown to be 100% efficacious in preventing malaria in children and a study in Kenya, where Malarone was shown to be 100% efficacious in preventing malaria in adults. Two subjects developed parasitemia after six weeks of treatment. In both subjects, the mean plasma concentrations of atovaquone at week 5 were within the range of concentrations of those in subjects who did not develop malaria. However, the plasma concentrations of proguanil and cycloguanil at week 5 were not measurable in one subject and barely above the quantifiable limit in the other. The reason for low concentrations of proguanil and cycloguanil is unknown but this finding suggests that the synergistic activity between atovaquone and proguanil/cycloguanil is important not only for the treatment, but also for the prophylaxis of malaria. *In vitro* sensitivity testing was not performed on isolates from these two subjects and the results of DNA sequence analysis to look for evidence of mutations in the cytochrome b gene associated with resistance to atovaquone are not presently available.

The safety data from this study confirm that Malarone is well tolerated and, at prophylactic doses, the frequency of adverse events in the Malarone treatment group was not higher than in the placebo group. No treatment-emergent effects were evident for any of the clinical laboratory parameters measured.

The observation that all 20 subjects with parasitemia at baseline and a blood film at day 7 were cleared of infection during the radical cure phase also supports earlier findings that Malarone is a safe and efficacious therapeutic option for treating *P. falciparum* malaria. Thus, Malarone appears to have an excellent efficacy and safety profile for chemoprophylaxis against *P. falciparum* malaria for subjects who are exposed to infection in malaria-endemic areas.

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