

Prolonged Protection Provided by a Single Dose of Atovaquone-Proguanil for the Chemoprophylaxis of *Plasmodium falciparum* Malaria in a Human Challenge Model

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Background. We conducted a randomized, placebo-controlled, double-blind trial to establish the efficacy of atovaquone-proguanil to prevent malaria with the goal of simulating weekly dosing in a human *Plasmodium falciparum* challenge model.

Methods. Thirty volunteers randomly received 1 of the following dose regimens: (1) 250 milligrams of atovaquone and 100 milligrams of proguanil (250/100 milligrams) 1 day prior to infectious mosquito challenge (day -1), (2) 250/100 milligrams on day 4 after challenge, (3) 250/100 milligrams on day -7, (4) 500 milligrams of atovaquone and 200 milligrams of proguanil (500/200 milligrams) on day -7 or, (5) 1000 milligrams of atovaquone and 400 milligrams of proguanil (1000/400 milligrams) on day -7. All regimens included matching placebo such that all volunteers received identical pill numbers. Six volunteers served as open-label infectivity controls. Volunteers underwent mosquito sporozoite challenge with *P. falciparum* 3D7 strain. Follow-up consisted of serial microscopy and close clinical monitoring for 90 days.

Results. Six of 6 infectivity controls developed parasitemia as expected. Two of 5 evaluable volunteers receiving 250/100 milligrams 7 days prior to challenge and 1 of 6 volunteers receiving 1000/400 milligrams 7 days prior to challenge were microscopically diagnosed with malaria. All other volunteers were protected. Atovaquone exposure (area under the curve) during liver stage development was low in 2 of 3 volunteers with prophylactic failure (423 and 199 ng/mL × days compared with a mean for protected volunteers of 1903 ng/mL × days), as was peak concentration (165 and 81 ng/mL compared with a mean of 594 ng/mL in volunteers with prophylactic success). Elimination half-life was short in volunteers with prophylactic failure (2.4, 2.0, and 3.3 days compared with a mean of 4.1 days in volunteers with prophylactic success).

Conclusions. Single-dose atovaquone-proguanil provides effective malaria chemoprophylaxis against *P. falciparum* challenge at dosing intervals supportive of weekly dosing. Postexposure prophylaxis 4 days after challenge was 100% effective.

Each year, an estimated 30 million international travelers from nontropical countries are at risk of malaria [1]. Malaria is the most common cause of febrile illness in

travelers to tropical regions and resulted in more than 10 000 cases reported among residents of the United States from 1997 through 2006 [2, 3]. Failure to take or adhere to a recommended chemoprophylaxis regimen was a contributing factor in most of these cases [4]. Although weekly dosing of prophylactic medications might enhance adherence, only 3 drugs, chloroquine, hydroxychloroquine, and mefloquine, are approved for weekly administration to prevent malaria. Each of these options has limitations, such as widespread resistance to chloroquine

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and hydroxychloroquine and the perception of unfavorable tolerability for mefloquine. Additional chemoprophylactic drugs with weekly dosing schedules would be useful in the prevention of travel-associated malaria.

Atovaquone-proguanil (A-P; 250 milligrams of atovaquone and 100 milligrams of proguanil; brand name, Malarone, GSK, Middlesex, United Kingdom) is a synergistic combination drug approved by the US Food and Drug Administration (FDA) and European Medicines Agency for treatment and prophylaxis of *Plasmodium falciparum* malaria. The current approved regimen of A-P for the prevention of malaria is 1 tablet daily beginning prior to departure and continuing during the period of exposure, and for 7 days on return. The reported elimination half-life of atovaquone is 2–3 days [5], although this was primarily determined in subjects weighing <60 kilograms [6]. A report of 3 Australian volunteers determined the half-life to be 5.9 days [7]. That of proguanil is 12–21 hours and similar for the active metabolite, cycloguanil [5, 8]. Repeated studies have shown that after a treatment course (4 tablets daily for 3 days) of A-P, individuals are protected from re-infection with *P. falciparum* for 28–32 days [9–12]. A dose of 250 milligrams of atovaquone protected 6 of 6 of volunteers who were dosed 1 day prior to sporozoite challenge [13]. Similarly, proguanil protected healthy volunteers after various single doses of proguanil (10–100 milligrams) given 2–5 days after *P. falciparum* sporozoite challenge [14]. Taken together, these results suggest that daily dosing of A-P may not be necessary for effective *P. falciparum* malaria protection.

In order to assess the potential for weekly dosing of A-P for malaria prophylaxis, we conducted a clinical trial utilizing the human sporozoite challenge model, which allows for reliable infection with controlled timing [15]. We assessed the efficacy of A-P at time points relative to infection relevant to weekly dosing for prophylaxis.

METHODS

Study Volunteers

This study was conducted in accordance with the Declaration of Helsinki (World Medical Association, 1964, amended 2008) under a protocol reviewed and approved by the Walter Reed Army Institute of Research (WRAIR) institutional review board (IRB) as well as by the US Army Medical Research and Materiel Command Human Subjects Protection Office. The protocol was conducted under an investigational new drug application and reviewed by the US FDA prior to execution. It was registered at its inception with [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT00984256). Written informed consent was obtained from all potential participants prior to screening and enrollment.

Through IRB-approved advertisements, 36 volunteers were recruited. Volunteers consented to participate in either the control cohort or the prophylaxis cohort according to personal

preference. Volunteers included men and nonpregnant, non-lactating women 18–50 years of age who were available to participate for the duration of the study. All volunteers were required to score >80% on a quiz of understanding of risks and study procedures. Volunteers were excluded if they had any history of malaria or travel within the past 12 months to a country with malaria transmission. Additional exclusion criteria included use of concomitant medications with antiplasmodial activity or immunosuppressant medications; clinically significant abnormalities on medical history, physical examination, laboratory testing, electrocardiogram, sickle cell disease, or trait; cardiac risk score of greater than low risk on the scoring system by Gaziano et al [16]; or a body mass index (BMI) of <19 or >30. Members of the infectivity control cohort were not subject to BMI restrictions as they did not undergo drug dosing.

Study Design

Volunteers for the control cohort were enrolled in an open-label study and did not undergo randomization. Volunteers for the prophylaxis cohort were randomly assigned to 1 of 5 dose groups. Group 1 received a single dose of 250 milligrams of atovaquone and 100 milligrams of proguanil (250/100 milligrams) the day prior to challenge (day –1). Group 2 received a single dose of 250/100 milligrams 4 days after challenge (day 4). Groups 3, 4, and 5 received single doses of 250/100 milligrams, 500 milligrams of atovaquone and 200 milligrams of proguanil (500/200 milligrams), or 1000 milligrams of atovaquone and 400 milligrams of proguanil (1000/400 milligrams) 7 days prior to challenge (day –7), respectively (Figure 1). Matching placebo was used in all groups, and all volunteers and study personnel were blind to group assignment. This study was conducted from September 2009 through the end of January 2010 at the WRAIR Clinical Trials Center, Silver Spring, Maryland.

Chemoprophylaxis

Gelatin capsules were filled either with A-P (Malarone, GSK, Middlesex, United Kingdom) 250/100 milligrams tablets or with lactose powder. Preparation of unit doses and randomization were performed by the investigational pharmacy of the Walter Reed Army Medical Center (Washington, D.C.). All drug doses were administered under direct observation with a snack containing ~11 grams of fat.

Malaria Sporozoite Challenge

On day 0 of the study, all participants underwent a malaria sporozoite challenge with *P. falciparum* parasites (strain NF54, clone 3D7) following a standardized challenge model that has been well described elsewhere [15]. In vitro susceptibility to standard anti-malarial drugs was verified for this isolate prior to challenge. The 50% inhibitory concentration (IC₅₀) for chloroquine was 3.3 ng/mL (susceptible), that for proguanil was 362 ng/mL (susceptible), and that for atovaquone was 0.19 ng/mL (susceptible).

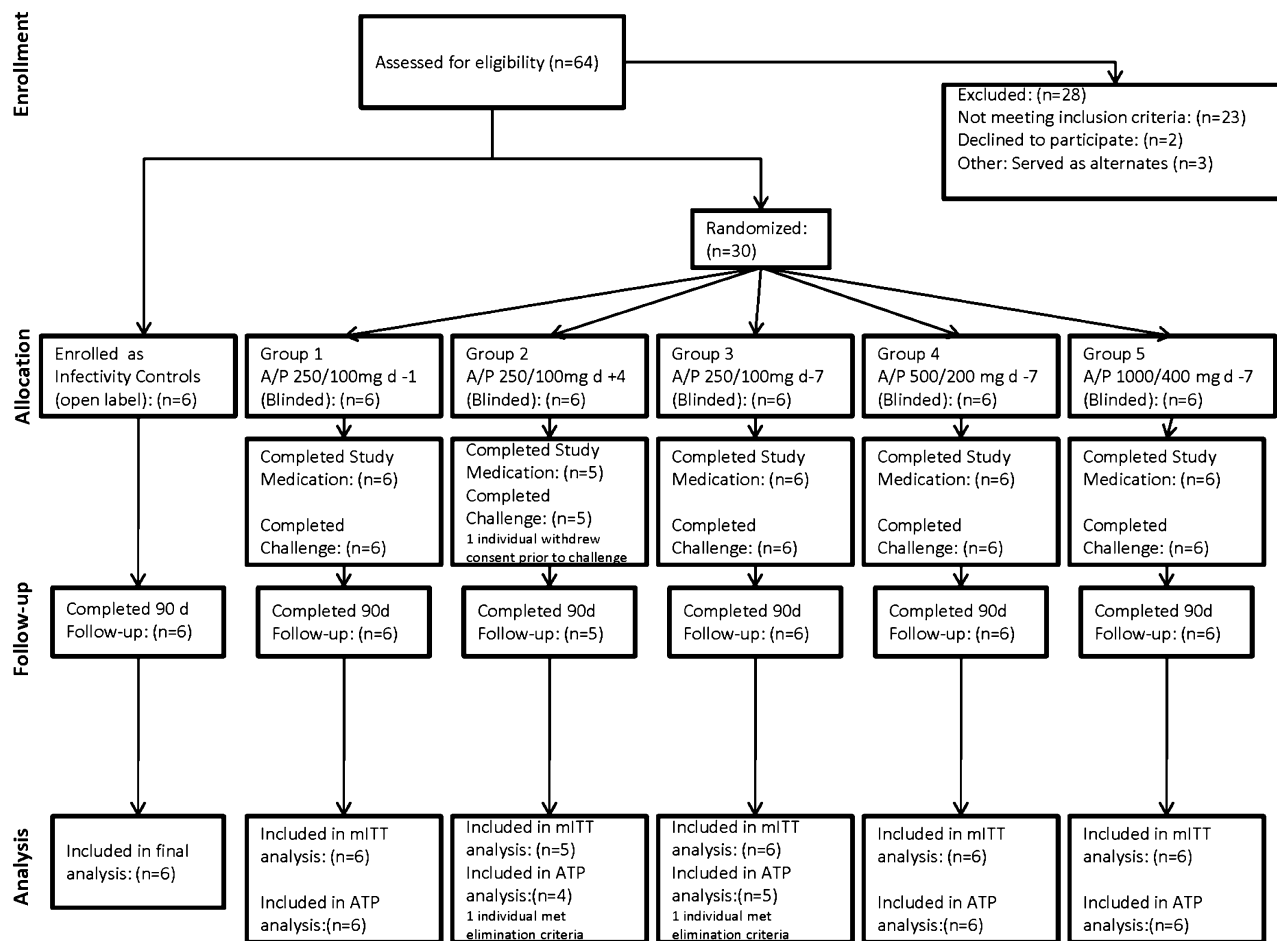


Figure 1. Study flow diagram. Abbreviations: A-P, atovaquone-proguanil; ATP, according to protocol; mITT, modified intention to treat; 250/100 mg, 250 milligrams of atovaquone and 100 milligrams of proguanil; 500/200 mg, 500 milligrams of atovaquone and 200 milligrams of proguanil; 1000/400 mg, 1000 milligrams of atovaquone and 400 milligrams of proguanil.

Parasites were thawed and expanded from a master seed lot. They were then used to infect laboratory-born and reared *Anopheles stephensi* mosquitoes. During the actual challenge, groups of 5 mosquitoes were allowed to feed for 5 minutes upon the forearm of each volunteer, then dissected and their salivary glands scored to ensure the presence of a sufficient density of sporozoites by a standardized scoring system. If required, additional mosquitoes were allowed to feed until a total of 5 mosquitoes with an appropriate sporozoite density had fed on each volunteer.

Assessment of Efficacy and Safety

Volunteers were assessed as outpatients on days -7, -6, -5, -1, 0, 1, 4-20, 23, 28, 42, 70, and 90. From days 9 through 20, volunteers were asked to spend nights at a study hotel to allow for closer observation during the period of greatest risk for parasitemia. Prophylactic success was defined as absence of parasitemia on Giemsa-stained thick blood film throughout the study period. Daily blood samples for thick blood film were obtained on days 6-20 and day 23 and at visits for febrile illness

throughout the 90-day observation period. Samples for polymerase chain reaction (PCR) were obtained at corresponding time points, although these were not read in real time. Individuals determined to be parasitemic on Giemsa-stained thick blood films were treated with 1 gram of chloroquine initially followed by 500 milligrams in 6-12 hours and daily for 2 days. They were followed up with daily blood films until 3 consecutive samples confirmed clearance of parasitemia.

Safety was assessed at each clinic visit with the question "Have you felt different in any way since your last visit?" followed by prespecified questions about specific symptoms. Investigators determined that adverse events (AEs) were attributed to study participation on a scale ranging from "not related" to "definitely related". AEs were attributed to either "prophylaxis medication," "treatment medication," "mosquito challenge," "Plasmodium infection," "other," or "cannot be determined" and graded in severity according to the Common Terminology Criteria for Adverse Events, version 4.01 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

Laboratory Methods

Trained microscopists reviewed a minimum of 200 oil immersion fields of each thick blood film. All parasites were confirmed by a second, expert reviewer.

A full description of methods used for drug susceptibility testing, plasma drug concentration determinations, and PCR can be found in the Appendix. Pharmacokinetic analysis was performed in SAS, version 9.2 (SAS Institute, Cary, NC). Drug exposure was determined according to the simple trapezoidal rule and presented in units of ng/mL × days.

Statistical Analyses

Descriptive and statistical analyses were performed using SAS, version 9.2, and SPSS, version 16 (IBM, Somers, NY). Defined populations for analysis were “safety,” which included all enrolled volunteers; “modified intention to treat”, which included all volunteers who underwent dosing and challenge; and “according to protocol” (ATP), which included those participants meeting all eligibility criteria, not meeting any elimination criteria, complying with the procedures defined in the protocol, and for whom data are available.

RESULTS

Volunteers

Six volunteers enrolled in the control cohort. Thirty volunteers enrolled into the prophylaxis cohort were randomly assigned evenly to 5 groups. Demographic characteristics are shown in Table 1. A single volunteer from group 2 withdrew consent prior to challenge.

Efficacy

Six of 6 volunteers assigned to the control cohort developed parasitemia, confirming the infectivity of the challenge procedures. Parasitemia in the control volunteers was detected 9–13 days after challenge, consistent with experience from other malaria challenge studies [17]. One volunteer each from groups 2 and 3 were excluded from the ATP analysis for meeting exclusion criteria; specifically, each took an antibiotic with antiparasitodal activity (doxycycline and azithromycin, respectively) during the follow-up period. Each of these volunteers had not developed parasitemia for >50 days before taking these medications. From the prophylaxis cohort, 3 volunteers developed

Table 1. Demographic Characteristics of Members of the Modified Intent to Treat Study Population

Variable	Control group (N = 6)	Group 1 (N = 6)	Group 2 (N = 5)	Group 3 (N = 6)	Group 4 (N = 6)	Group 5 (N = 6)	Overall (N = 35)
Sex							
Male	5 (83)	5 (83)	1 (20)	5 (83)	3 (50)	4 (67)	23 (66)
Female	1 (17)	1 (17)	4 (80)	1 (17)	3 (50)	2 (33)	12 (34)
Age, years							
Mean (SD)	28.3 (5.0)	35.7 (9.4)	31.2 (8.2)	30.5 (10.5)	31.8 (11.8)	29.6 (7.3)	31.2 (8.6)
Range	24–38	22–48	24–44	20–49	21–50	21–38	20–50
Height, in							
Mean (SD)	70.7 (4.0)	66.8 (3.5)	66.2 (4.4)	68.3 (2.0)	69.8 (3.7)	68.5 (4.8)	68.5 (3.9)
Range	64–75	63–71	61–73	67–72	67–75	63–75	61–75
Weight, kg							
Mean (SD)	85.5 (13.4)	67.9 (9.6)	74.6 (13.2)	81.3 (8.2)	78.7 (15.5)	76.6 (13.4)	77.5 (12.8)
Range	75.3–109.3	52.6–81.6	63.5–95.7	65.3–88	56.7–103.9	51.3–86.6	51.3–109.3
BMI, kg/m²							
Mean (SD)	27.0 (5.2)	23.8 (2.9)	26.4 (3.3)	27.0 (3.2)	25.0 (3.4)	25.3 (3.3)	25.7 (3.6)
Range	22–36	20–28	22–30	22–30	20–30	20–30	20–36
Ethnicity							
Hispanic or Latino	0 (0)	0 (0)	2 (40)	1 (17)	0 (0)	0 (0)	3 (9)
Non-Hispanic or Non-Latino	6 (100)	6 (100)	3 (60)	5 (83)	6 (100)	6 (100)	32 (91)
Race							
American Indian or Alaska Native	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	1 (3)
Asian	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)	1 (3)
Black or African American	3 (50)	4 (67)	1 (20)	4 (67)	3 (50)	3 (50)	18 (51)
Native Hawaiian or Pacific Islander	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
White/Caucasian	3 (50)	2 (33)	3 (60)	2 (33)	3 (50)	2 (33)	15 (43)

Data are no. (%) of participants, unless otherwise indicated. Group 1 received a single dose of 250 milligrams of atovaquone and 100 milligrams of proguanil (250/100 mg) the day prior to challenge (day –1). Group 2 received a single dose of 250/100 mg 4 days after challenge (day 4). Groups 3, 4, and 5 received single doses of 250/100 mg, 500 milligrams of atovaquone and 200 milligrams of proguanil, or 1000 milligrams of atovaquone and 400 milligrams of proguanil 7 days prior to challenge (day –7), respectively.

Abbreviations: BMI, body mass index; kg, kilograms; SD, standard deviation.

malaria, 2 in group 3 and 1 in group 5. In the ATP analysis, 6 of 6 individuals in group 1 were protected from malaria for the duration of the study and were thus considered to have prophylactic success, as were 4 of 4 from group 2, 3 of 5 from group 3, 6 of 6 from group 4, and 5 of 6 from group 5.

For 1 individual from the control cohort with microscopically detected parasitemia, PCR from corresponding time points was negative. The individual from group 5 with microscopically detected parasitemia did not have a corresponding PCR sample but had a negative PCR result 24 hours before. All other PCR results corresponded to microscopy results. In 2 cases, PCR and microscopy results became positive on the same day. In 2 cases, microscopic detection of parasites preceded PCR positivity by 24 hours, whereas in 2 more cases, PCR positivity preceded microscopic detection by 24 hours, and in a single case, PCR positivity occurred 48 hours before microscopic detection. The mean quantitative result for initial positive PCR samples was 2.7 parasites/ μ L (SD, 1.5 parasites/ μ L). Human beta-actin was detected to assure successful DNA preparation.

Of the 3 prophylaxis failures, only the 2 from group 3 had cultivable parasites. In these 2 individuals, there was no evidence of reduced drug susceptibility. The IC_{50} of atovaquone from clinical samples from volunteers 1 and 23 was 0.15 ng/mL (SD, 0.05 ng/mL) and 0.29 ng/mL (SD, 0.23 ng/mL), respectively, compared with 0.19 ng/mL in the parent strain. For comparison, a threshold of 7.3 ng/mL has been used to identify strains resistant to atovaquone [18]. The IC_{50} of proguanil was also unchanged in samples from these volunteers, with mean values of 252 ng/mL (SD, 90 ng/mL) from volunteer 1 and 473 ng/mL (SD, 215 ng/mL) from volunteer 23 compared with 362 ng/mL for the parent strain.

Pharmacokinetics

There was considerable individual variability in all pharmacokinetic parameters of atovaquone, proguanil, and cycloguanil (Table 2). By linear regression, there was a relationship between maximum concentration (C_{max}) and BMI ($R^2 = 0.301$; $P = .03$) and between drug exposure (area under the concentration curve [AUC]) and weight ($R^2 = 0.273$; $P = .05$) among the combined volunteers from groups 1, 2, and 3 who all received the same dose of A-P, although these relationships were not responsible for the majority of the variability observed.

Compared with prophylactic successes, prophylactic failures had a nonsignificant trend toward lower atovaquone drug exposure ($AUC_{0-6.5}$, 1903 vs 973 ng d/mL) and maximum plasma concentration ($C_{max 0-6.5}$, 594 vs 280 ng/mL) during liver stage development. Two of the 3 individuals with failed prophylaxis had values of $C_{max 0-6.5}$ and $AUC_{0-6.5}$ that were among the lowest in the prophylaxis cohort (Figure 2).

We observed considerable individual variation in the elimination half-life ($T_{1/2}$) of atovaquone. Although small sample size limits statistical comparison, there was a trend toward shorter $T_{1/2}$ among prophylactic failures than among successes, with medians of 2.37 days (range, 2.0–3.3 days) compared with 3.22 days (range, 1.7–11.0 days), respectively. Two of the prophylaxis failures were in those individuals in the lowest dose group (group 3) who had the fastest elimination half-lives (Figure 3).

Plasma concentrations of proguanil were low by the time of challenge in groups dosed on day -7, with median $C_{max 0-6.5}$ values below the limit of detection, of 0.76 ng/mL, and of 1.66 ng/mL in groups 3, 4, and 5 respectively.

Table 2. Atovaquone Pharmacokinetic Parameters According to Protocol Population

Variable	Group 1 (N = 6)	Group 2 (N = 4)	Group 3 (N = 5)	Group 4 (N = 6)	Group 5 (N = 6)	Overall (N = 27)
AUC_{overall}, ng/mL × days						
Mean (SD)	4624 (2693)	3066 (1674)	2668 (679)	5515 (1304)	10,319 (4744)	...
Median (range)	4765 (1264–7780)	3062 (1362–4779)	2819 (1492–3228)	5898 (3579–6743)	8944 (6386–19,348)	...
AUC_{0-6.5}, ng/mL × days						
Mean (SD)	3595 (2213)	616 (191)	510 (218)	1434 (664)	2233 (1895)	1799 (1756)
Median (range)	3744 (1013–7267)	637 (385–803)	507 (199–763)	1314 (758–2321)	1701 (802–5824)	1013 (199–7267)
C_{max overall}, ng/mL						
Mean (SD)	931 (513)	728 (234)	634 (200)	834 (232)	1993 (369)	...
Median (range)	847 (299–1735)	763 (429–957)	689 (348–821)	808 (545–1136)	2068 (1489–2456)	...
C_{max 0-6.5}, ng/mL						
Mean (SD)	931 (513)	728 (234)	150 (39)	354 (92)	619 (440)	559 (418)
Median (range)	847 (299–1735)	763 (429–957)	165 (80–177)	339 (260–469)	508 (230–1470)	429 (81–1735)
T_{1/2}, days						
Mean (SD)	3.3 (1.9)	3.3 (1.2)	3.3 (1.6)	5.6 (3.1)	3.7 (1.4)	3.9 (2.1)
Median (range)	2.9 (1.7–7.0)	3.4 (2.0–4.6)	2.4 (2.0–5.8)	5.0 (2.4–11.0)	3.2 (2.3–5.5)	3.2 (1.7–11.0)

Group 1 received a single dose of 250 milligrams of atovaquone and 100 milligrams of proguanil (250/100 mg) the day prior to challenge (day -1). Group 2 received a single dose of 250/100 mg 4 days after challenge (day 4). Groups 3, 4, and 5 received single doses of 250/100 mg, 500 milligrams of atovaquone and 200 milligrams of proguanil, or 1000 milligrams of atovaquone and 400 milligrams of proguanil 7 days prior to challenge (day -7), respectively.

Abbreviation: AUC, area under the concentration curve; C_{max} , maximum plasma concentration; SD, standard deviation; $T_{1/2}$, elimination half-life.

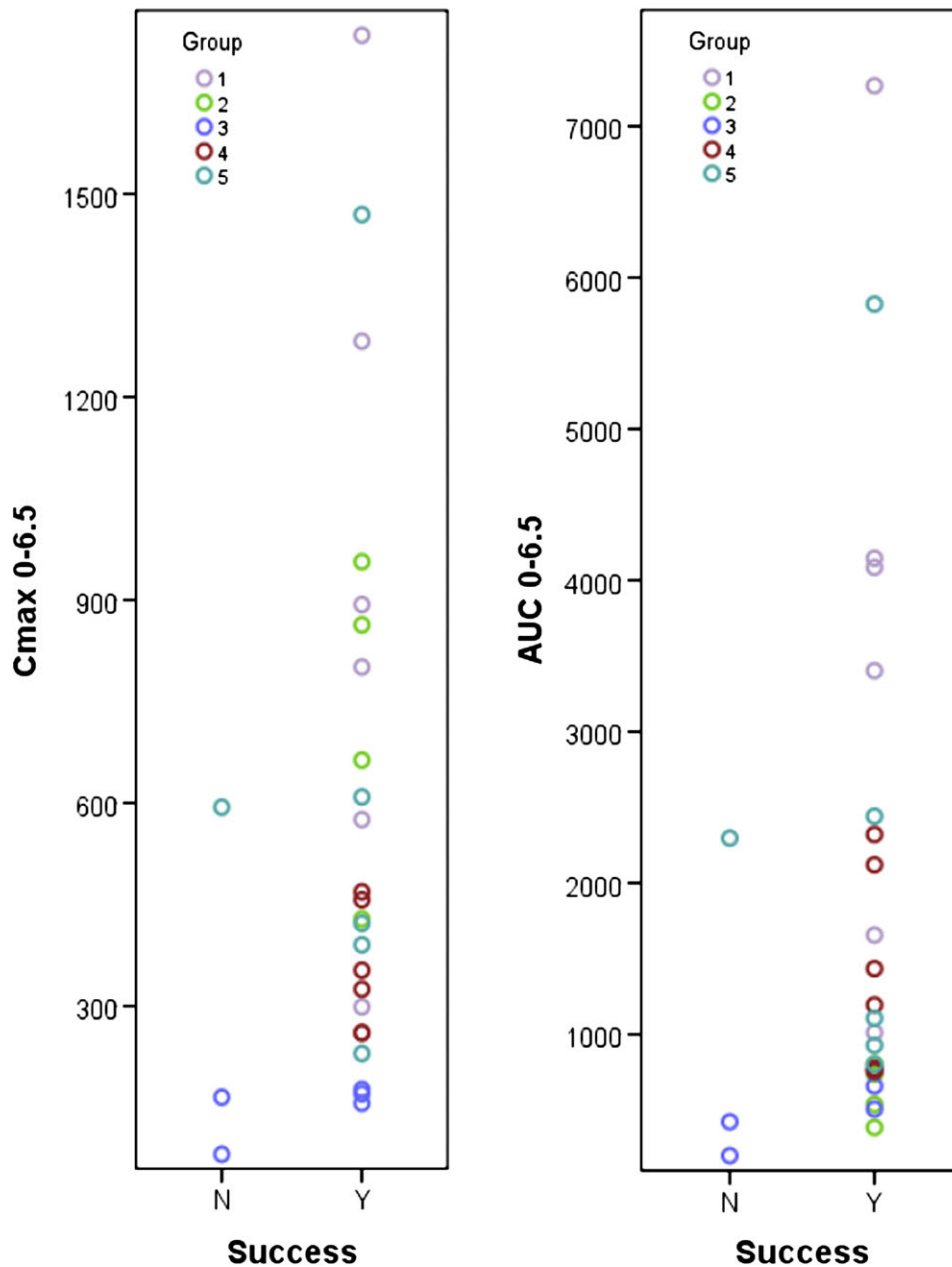


Figure 2. Peak concentration ($C_{\max 0-6.5}$, expressed in nanograms per milliliter) and area under the concentration curve ($AUC_{0-6.5}$, expressed in $\text{ng/mL} \times \text{days}$) of atovaquone plasma concentrations between study day 0 to study day 6.5, corresponding to the period of expected liver-stage development. Results are separated by prophylaxis success (Y) or failure (N).

Safety

A-P was well tolerated by all study participants. Overall, the most frequently occurring AEs were headache, pruritus, and upper respiratory tract infections. AEs attributed as possibly or probably due to A-P were 2 episodes headache (mild), 2 episodes of nausea (mild), and 1 episode of dry mouth (mild).

DISCUSSION

A single dose of A-P at times relevant to weekly dosing intervals was highly efficacious at protecting individuals from challenge

with *P. falciparum*. Results from group 1 confirm and build on those of Shapiro et al [13], who achieved 100% protection with 250 milligrams of atovaquone dosed 1 day before challenge. Similarly, results from group 2, dosed 4 days after challenge, were expected based on results generated by Fairley et al [14] using proguanil alone. Dose timing in the present study presented a greater challenge for the drug regimen than would weekly use because each experimental arm received only a single dose. Each would have been scheduled to receive additional doses either before or after challenge if dosed on a weekly schedule. For

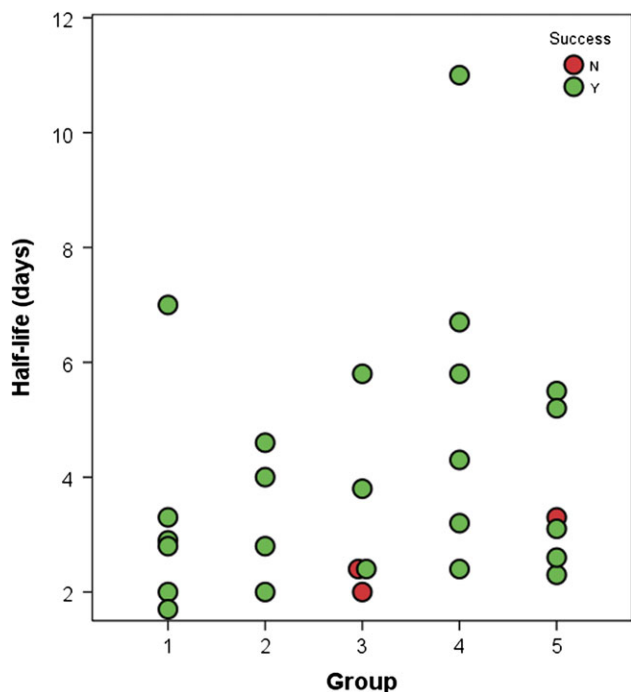


Figure 3. Scatterplot depicting plasma elimination half-life of atovaquone by study group. Red circles indicate prophylactic failures, and green circles indicate prophylactic successes.

groups 3–5, dosed on day –7, weekly dosing would result in additional doses on days 0 and 7. Efficacy despite this schedule suggests that daily dosing of A-P might have a sufficient margin of safety to allow for inadvertently late or missed doses and also supports the concept of weekly dosing. Furthermore, it raises questions about the need to complete 7 days of dosing after travel as is required by the current product label.

The relative contributions of atovaquone and proguanil to the observed prophylactic efficacy are unclear. Results from the groups dosed on day –7, in which there was little exposure to proguanil or cycloguanil, suggest that atovaquone is the predominant contributor to efficacy. Groups dosed on day 1 before or day 4 after challenge, which did have exposure to both atovaquone and proguanil during liver stage development, had 100% protection. It is possible that this combined exposure contributed to the high degree of protection observed.

Although excluded from the ATP analysis as specified in the protocol, we find it biologically implausible that the antibiotic medications taken by the 2 eliminated volunteers >50 days after challenge contributed to their successful prophylaxis. It seems likely that these volunteers were successfully protected by the prophylaxis regimens. Another uncertainty in the results relates to the volunteer from group 5 who was considered to have a prophylaxis failure (volunteer 26). This volunteer presented with symptoms atypical of malaria, including a sore throat. Although parasites were detected on microscopy at a single time

point on day 21 after challenge, attempts to culture parasites were negative as was PCR from day 20 (the closest time point to diagnosis available for analysis). Although microscopic parasitemia was detected according to study procedures, given the history of imperfect specificity of microscopy in clinical trials, these results call into question whether this volunteer truly had malaria [19]. If this result was due to false-positive microscopy, then pharmacokinetic parameters might have been even more predictive of prophylactic success than observed in our analysis. This uncertainty highlights difficulties inherent in the use of microscopic endpoints in trials of malaria chemoprophylaxis. In future trials, consideration should be given to use of endpoints incorporating compatible clinical symptoms, or alternate diagnostic tests in conjunction with microscopy.

The wide heterogeneity of pharmacokinetic results was surprising but not inconsistent with previously published reports of atovaquone pharmacokinetics [7]. Atovaquone absorption is known to be affected by food and particularly enhanced by ingestion of fat prior to dosing [20]. We attempted to minimize this effect by dosing A-P with food with a defined fat content under direct observation. Despite these measures, there was considerable individual variability in both C_{max} and AUC of atovaquone. This variation could not be fully explained by differences in weight or BMI. The relationship observed between prophylactic efficacy and drug exposure during liver stage development is intuitively logical and allows for pharmacokinetic modeling to predict dosing regimens that would be expected to retain efficacy.

This study has several limitations. The human challenge utilized a single parasite strain with known A-P drug sensitivity. Presumably if an A-P-resistant strain was encountered, the results would not be so favorable. Because the Y268S mutation responsible for most clinical cases of atovaquone resistance results in a several thousand-fold increase in IC_{50} , it is likely that successful prophylaxis with A-P of any parasite harboring this mutation might be dependent on proguanil exposure [21, 22]. It is possible that a prolonged dosing interval might decrease efficacy against these strains due to subtherapeutic proguanil concentrations during portions of the dosing interval. Although the sporozoite inoculum used in this challenge model probably exceeds that encountered in most field exposures, intensity of exposure is limited by the single exposure. Perhaps as a result of these limitations, the human malaria challenge model in the past may have overestimated prophylactic drug efficacy compared with drug performance in field trials [23, 24]. Confirmation of these results in field studies would be required before their applicability to clinical practice can be determined. As discussed, reliance exclusively on microscopic endpoints creates a limitation that may have affected our results. In addition, the small numbers of prophylaxis failures limited our ability to statistically detect pharmacokinetic differences between prophylactic successes and failures.

In summary, a single dose of A-P demonstrated prophylactic efficacy at time points relevant to weekly dosing schedules and postexposure prophylaxis in a human malaria challenge. These results require confirmation in larger field trials but suggest that A-P may have a sufficient therapeutic margin of safety to allow for weekly dosing or provide a measure of safety in the event of inadvertently missed doses without a decrease in prophylactic efficacy.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. All authors have contributed to the design or execution of this trial and have participated in the preparation of this manuscript.

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