Determinants of Treatment Response to Sulfadoxine-Pyrimethamine and Subsequent Transmission Potential in Falciparum Malaria

Fabián Méndez1,2, Álvaro Muñoz3, Gabriel Carrasquilla4,5, Diana Jurado6, Myriam Arévalo-Herrera6, Joseph F. Cortese7, and Christopher V. Plowe7

1 Secretaría Departamental de Salud del Valle, Cali, Colombia.
2 Escuela de Salud Pública, Universidad del Valle, Cali, Colombia.
3 Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.
4 Departamento de Microbiología, Universidad del Valle, Cali, Colombia.
5 Fundación para la Educación Superior, Cali, Colombia.
6 Instituto de Inmunología, Universidad del Valle, Cali, Colombia.
7 Malaria Section, Center for Vaccine Development, University of Maryland, Baltimore, MD.

Received for publication September 5, 2001; accepted for publication March 15, 2002.

Drug resistance is contributing to increasing mortality from malaria worldwide. For assessment of the role of resistance-conferring parasite mutations on treatment responses to sulfadoxine-pyrimethamine (SP) and transmission potential, 120 subjects with uncomplicated falciparum malaria from Buenaventura, Colombia, were treated with SP and followed for 21 days in the period February 1999 to May 2000. Exposures of interest were mutations in Plasmodium falciparum dihydrofolate reductase (DHFR) and dihydropteroate synthase that confer resistance to pyrimethamine and sulfadoxine, respectively. Although SP was highly efficacious (96.7%), the presence together of DHFR mutations at codons 108 and 51 was associated with longer parasite clearance time (relative hazard = 0.24, \( p = 0.019 \)) more so than the 108 mutation alone (relative hazard = 0.45, \( p = 0.188 \)). This association remained after controlling for potential confounders. Infections with these mutations were also associated with the presence of gametocytes, the sexual form of the parasite responsible for transmission, 14 and 21 days after treatment (\( p = 0.016 \) and \( p = 0.048 \), respectively). Higher gametocytemia is probably due to DHFR mutations prolonging parasite survival under drug pressure, resulting in longer parasite clearance time and allowing asexual parasites to differentiate into gametocytes. These results suggest that even when SP efficacy is high, DHFR mutations that are insufficient to cause therapeutic failure may nevertheless increase malaria transmission and promote the spread of drug resistance. *Am J Epidemiol* 2002;156:230–8.

dihydropteroate synthase; drug resistance; malaria; Plasmodium falciparum; pyrimethamine; sulfadoxine

Abbreviations: DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; PCT, parasite clearance time; SP, sulfadoxine-pyrimethamine; WHO, World Health Organization.

Much of the increasing morbidity and mortality caused by *Plasmodium falciparum* malaria is the result of resistance to the current antimalarial repertoire and the lack of affordable alternatives (1). The antifolate combination sulfadoxine-pyrimethamine (SP) has been a mainstay in efforts to combat chloroquine-resistant falciparum malaria, but resistance to SP itself arises rapidly in areas where its use is extensive (2). In South America, chloroquine failure is almost ubiquitous, and antifolate resistance has reached very high levels through much of the Amazon, such that neither chemotherapy is consistently effective, and in some areas, both treatments are proscribed (3). On the Pacific coast of Colombia, chloroquine resistance is present at the high levels characteristic of much of South America, but SP
resistance has remained low. However, as SP becomes the standard treatment, monitoring efforts to detect the emergence of resistance are needed.

The molecular mechanisms of antifolate resistance have been clearly defined in vitro. Point mutations in the *P. falciparum* genes encoding dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) confer resistance to pyrimethamine and sulfadoxine, respectively (4–8). These resistance-conferring mutations occur in a stepwise, sequential fashion, with higher levels of in vitro resistance occurring in the presence of multiple mutations. However, clinical SP failure is an outcome of a complex interplay of host, parasite, and drug, and identification of specific sets of mutations predictive of clinical resistance has been difficult (1, 9). An ecologic analysis found increasing prevalence of DHFR and DHPS mutations in four countries with increasing levels of SP resistance (3), and various sets of DHFR and DHPS mutations have been associated with SP failure in settings with relatively high levels of SP therapeutic failure (10, 11). Recently, a set of five DHFR mutations and two DHPS mutations was shown to be strongly associated with SP failure in Malawi (12), and new models that account for host factors contributing to parasite clearance may permit use of these mutations as molecular markers for drug resistance surveillance (13). However, the effects of parasite mutations on treatment outcomes in settings of low antifolate resistance are less well understood, and the factors involved in the vector-host transmission of drug resistance remain imprecisely defined.

In this study of 120 subjects from the Pacific coast of Colombia, we assessed the association between the occurrence of mutations in *P. falciparum* DHFR and DHPS genes with SP treatment response and also with subsequent development of gametocytemia (carriage of the sexual forms of the parasite required for transmission by the mosquito). This study sought to contribute to an understanding of the molecular basis of treatment response to SP and to explore the impact of DHFR and DHPS mutations on transmission potential.

**MATERIALS AND METHODS**

**Study population and methods**

The study was conducted from February 1999 to May 2000 in Buenaventura, a seaport on the Pacific coast of Colombia with hypoendemic malaria transmission. Subjects were recruited from outpatient clinics, and treatment outcomes were measured using a standard World Health Organization (WHO) protocol (14). Subjects were eligible for enrollment if they were aged 1 year or more, had *P. falciparum* monoinfection with a parasitemia of 1,000–100,000 parasites/µl, reported or had documented fever during the current episode of malaria, had a temperature of less than 39.5°C, and gave informed consent. Exclusion criteria included signs of severe malaria according to WHO guidelines (15), pregnancy, febrile conditions caused by diseases other than malaria, and inability to stay in the study area for the 21-day follow-up period.

Standard SP treatment was administered under direct observation as a single dose of 1.25 mg pyrimethamine and 25 mg sulfadoxine per kg of body weight (maximum dose, 75 mg pyrimethamine and 1,500 mg sulfadoxine). Fever was treated with acetaminophen. Subjects who developed SP treatment failure were hospitalized, treated with intravenous quinine and/or amodiaquine, and followed until full recovery. All subjects were examined on posttreatment days 1, 2, 3, 7, 14, and 21 for determination of parasitemia and a clinical assessment including measurement of body temperature. Persons whose infections had not cleared on day 3 were seen the following day to monitor treatment response status. Patients were encouraged to come to the clinic at any time for any medical complaint.

**Exposures of interest**

The primary exposures of interest in this study were mutations in the parasite genes encoding DHFR and DHPS. Other exposures included drug concentrations, nutritional status, and potential confounders.

**Assessment of parasite mutations.** Finger-prick blood was collected on filter paper strips (Whatman, Hillsboro, Oregon), air dried, stored in separate envelopes, and transported at room temperature. After the blood was stored for several weeks at 4°C, DNA was extracted from pieces of blood-impregnated filter paper approximately 3 mm² by using a methanol fixation-heat extraction method (16). Polymerase chain reaction protocols have been described in detail elsewhere (3) and are available on the Internet at http://medschool.umaryland.edu/CVD/plowe.html. Briefly, a nested, allele-specific polymerase chain reaction was performed for the analysis of each codon of interest. A pilot study conducted at the study site did not detect infections with mutations at DHFR codons 50 or 59 or at DHPS codon 581 (results not shown). Our analysis was therefore limited to the DHFR mutations at codons 108 (serine to asparagine), 51 (asparagine to isoleucine), and 164 (isoleucine to leucine) and the DHPS mutations at codons 437 (alanine to glycine) and 540 (lysine to glutamate).

**Assessment of drug concentrations.** Plasma was frozen in duplicate for determination of pyrimethamine and sulfadoxine concentrations 24 hours after treatment. Blood drug levels were determined using high performance liquid chromatography.

**Assessment of nutritional status.** We determined body mass index (weight (kg)/height (m²)) and percentiles of middle arm circumference and calculated z scores of the ratios weight:height and height:age by using the Centers for Disease Control and Prevention anthropometric software program Epi-nut (Centers for Disease Control and Prevention, Atlanta, Georgia). Standard criteria for age and gender limits of hematocrit and iron (17) were used to determine the presence of anemia.

**Other potential confounders.** At enrollment, we recorded age, gender, place of residence, report of microscopically diagnosed malaria during the preceding year, time from onset of illness to seeking treatment, and antimalarial drug intake prior to enrollment.
Outcome measures

Treatment outcomes. We used two measures of treatment response: incidence of treatment failure and parasite clearance time (PCT). Treatment outcome was classified as adequate clinical response or early or late treatment failure, using standard WHO criteria (14). PCT was defined as the time from starting antimalarial treatment until parasites were undetectable in the peripheral blood film. An experienced laboratory technician measured asexual parasitemia (asexual parasites/µl blood) by reading 200 high-power fields on Giemsa-stained thick blood films.

Transmission potential. We evaluated the potential for transmission by measuring gametocytemia on enrollment and at scheduled visits following the same technique used for asexual parasitemia.

Statistical methods

We calculated the proportion of malaria infections with mutations of interest present at the time of enrollment and estimated 95 percent binomial confidence intervals for prevalence of mutations in the study area. Since PCT was assessed at 24-hour intervals, a survival analysis for discrete time data (days to parasite clearance after treatment) was carried out using log-rank tests to compare PCT in persisting infections with or without each mutation of interest. This analysis is equivalent to the exact Mantel-Haenszel test or the score statistic of a Cox regression using exact likelihood methods (18). Extension of the methods for the analysis of several factors results in a semiparametric regression to estimate relative hazards and their 95 percent confidence intervals (18). The exact partial likelihood method was used to treat ties. With this method, the likelihood is changed to reflect the discreteness of time measures, and the conditional probability that the observed events are the ones that occurred in the risk pool given the observed number of events is calculated (18).

Subjects were classified by categories of putative determinants of treatment response (e.g., anemic or not, quartiles of initial parasitemia). To evaluate the role of potential determinants of treatment response as confounders, we characterized their relation with both the exposure (mutations) and the outcome (PCT). Based on results of the univariate analysis and previous knowledge of determinants of treatment response, a multiple Cox regression model was used to obtain the association of mutations and PCT adjusted by other confounders (18).

We used two descriptors for the distribution of gametocytes: 1) the proportion of persons with detectable gametocytes at each scheduled visit (percentage greater than zero or discrete component), and 2) mean gametocytemias (in the log10 scale), excluding those with no gametocytes (mean for those that are greater than zero or continuous component). Chi-square tests for trend were conducted to evaluate the dose-response relation between mutations and gametocytemia by each day of follow-up after treatment. Evaluation of significant differences for means of gametocytemias was carried out by linear regression.

RESULTS

Between February 1999 and May 2000, 120 subjects were enrolled in the study, 103 (85.8 percent) of whom completed follow-up and had a measurable treatment outcome. The 17 subjects with incomplete follow-up were examined for the last time either on day 14 (14/120 = 11.7 percent) or on day 7 (3/120 = 2.5 percent). Subjects were between ages 1 and 70 years (median = 21.2 years), 63 percent (76/120) were male, and 88 percent (106/120) lived in the urban study area.

Table 1 summarizes the prevalence of DHFR and DHPS mutations. Most of the samples (80 percent) that could not be amplified were more than 10 months old and/or had parasitemias below 5,000 parasites/µl. Mutations at DHFR codon 164 and DHPS codon 540 were not found in the analysis of the first 52 and 70 samples, respectively, and assays for these mutations were discontinued thereafter.

As expected, all infections with the DHFR 51 mutation also carried the serine to asparagine mutation at DHFR codon 108. Therefore, we categorized individual infections by their mutations at the DHFR codons as follows: no mutations (12/100), 108 mutant only (38/100), or 108 and 51 mutant (50/100). In addition, among persons with both of these DHFR mutations, 26 percent (13/50) also had the DHPS 437 mutation.

Determinants of treatment response

Four subjects developed treatment failure (4/120 = 3.3 percent), with one early and three late treatment failures. Figure 1 depicts the trajectories of log10 parasitemias for all
subjects, including the four treatment failures. To avoid ties in the graphic depiction, we randomly added a small quantity (between 0 and 0.5) to each value of parasitemia. The cumulative PCT distribution for the 120 study subjects was as follows: 24.2, 83.4, 95.8, and 96.7 percent cleared parasites by days 1, 2, 3, and 4, respectively. Subjects with a PCT of longer than 3 days \((n = 5)\) included four persons with treatment failure and one subject with adequate clinical response that cleared parasitemia on day 4.

**Mutations.** We carried out univariate analyses of the association between PCT and each DHFR and DHPS mutation of interest; subsequently, we determined the associations with multiple mutations. The presence of the DHFR 108 mutant alone delayed parasite clearance (relative hazard = 0.32, \(p = 0.04\)). The relative hazard for the DHFR 51 mutation was 0.52 (\(p = 0.076\)), consistent with an additive effect of this mutation, which occurs only in the presence of the mutation at codon 108. The DHPS 437 mutation was not significantly associated with PCT, showing a trend in the opposite direction (relative hazard = 1.83, \(p = 0.181\)).

The presence of mutations at both DHFR codons was more strongly associated with longer PCT (relative hazard for clearance = 0.24, \(p = 0.019\)) than that of the mutation at codon 108 alone (relative hazard = 0.45, \(p = 0.188\)) (table 2). Since the categorical analysis suggested a dose response, we carried out an analysis with a single variable, taking values 0, 1, and 2 for no mutations, 108 alone, and both the 108 and 51 mutations, respectively. With this continuous variable, the hazard ratio was 0.51 (\(p = 0.012\)), showing a significant trend of PCT being halved as an additional mutation is involved in the malaria infection.

**Drug concentrations, initial parasitemia, nutritional status, and other potential confounders.** An increase of 1 \(\mu g/ml\) in the pyrimethamine concentration was significantly associated with doubling the hazard rate of clearing parasitemia (relative hazard = 2.08, \(p = 0.041\)). A similar analysis for sulfadoxine did not show a significant association (relative hazard = 1.01, \(p = 0.397\)). Use of log10 parasitemia on day 0 as a continuous variable showed a significant trend to longer PCT with increasing values of parasitemia (\(p < 0.001\)). Persons with low iron plasma concentration tended to have a longer PCT (relative hazard = 0.36, \(p = 0.09\)). Low hematocrit, anthropometric indicators of poor nutritional status, age, gender, and previous malaria episodes all showed no significant association with PCT.

**Multivariate analysis of treatment response.** We evaluated confounding effects among the potential determinants of PCT. Because our primary interest was to evaluate parasite mutations as potential determinants of treatment response, we built a model selecting variables from factors that appeared to be important in the univariate analysis (pyrimethamine concentration, parasitemia on day 0, and low iron plasma concentration) or that had an important biologic association (age, gender, malaria episodes in the previous year, time to diagnosis, and chloroquine intake prior to therapy). We fitted a multiple Cox regression model for the variables that remained associated at \(p < 0.15\) (table 3). Inferences regarding the effect of mutations drawn from the univariate analysis remained after adjustment by levels of parasitemia and drug concentration.
Determinants of transmission potential

Figure 2 depicts the trajectories in log10 gametocytemias. Peak gametocytemia occurred 7–14 days after treatment, when 70 percent of subjects had detectable gametocytes. Mean log10 gametocytemias on days 7 and 14 were 2.75, corresponding to 562 gametocytes/µl (n = 82), and 2.45, corresponding to 282 gametocytes/µl (n = 72), respectively. On day 21, the proportion of gametocyte carriers decreased to 51 percent (49/96), and among the 49 gametocyte carriers, the mean of the log10 gametocytemia declined to 2.10, corresponding to 126 gametocytes/µl.

Mutations. Figure 3 shows the trajectories of proportions of gametocyte carriers by DHFR type. After a peak at day 7, infections with no DHFR mutations had a decrease in gametocyte carriage proportions (percent of infections with any gametocytes), while most mutant infections remained at a similar percentage of gametocytemia until day 14. Compared with infections with no mutations, those with both the 108 and 51 DHFR mutations had a larger increase in gametocyte carriage rates than did those with only the 108 mutation. Trends in proportions were statistically significant at days 14 (chi-square = 5.85, p = 0.016) and 21 (chi-square = 3.89, p = 0.048). In addition, comparisons of means of log10 gametocytemias (among those with gametocytemia > 0) showed a consistently higher level of gametocytemia in persons with mutant infections than in those whose infections lacked DHFR mutations. For example, at day 7, posttreatment mean gametocytemia of infections lacking
mutations (mean = 2.28 corresponding to 191 gametocytes/µl) was significantly lower than the mean gametocytemias of persons with only 108 mutations (mean = 2.92, corresponding to 832 gametocytes/µl, p = 0.004) or with both 108 and 51 mutations (mean = 2.83, corresponding to 676 gametocytes/µl, p = 0.010). The same trend was observed on days 14 and 21.

PCT. Persons with longer PCT were more likely to be gametocyte carriers: On day 14, half of the persons with PCT of 1 day developed a detectable gametocytemia, while more than 90 percent of those with PCT equal to 3 days presented gametocytes (p < 0.05 test for trend). Similarly, comparisons of means of log10 gametocytemias between persons with different PCT showed a consistently higher level of gametocytemia in persons with a longer PCT, and the differences were significant on day 7 (p < 0.05).

Multivariate analysis for gametocytemia. Using multiple logistic regression, we estimated the odds ratio for the association between mutations (as predictor variables) and the occurrence of gametocytemia, unadjusted and adjusted for duration of PCT (table 4). Because odds ratios of mutations tend to be closer to one when adjusted by duration of PCT, the results in table 4 indicate that part of the association of mutations and gametocytemia is indirect and can be explained by longer PCT. However, particularly at day 14, mutations at DHFR genes remained significantly associated with gametocytemia even after adjustment by duration of PCT. Results remained unchanged after further adjustment by asexual parasitemia at baseline.

DISCUSSION

In 1996, the Colombian Ministry of Health changed the standard treatment of uncomplicated falciparum malaria from chloroquine to SP in this area of Colombia. Our study shows that SP treatment remains highly efficacious on the Pacific coast of Colombia (96.7 percent efficacy, 95 percent confidence interval: 91.7, 99.1 percent). However, early treatment failures of P. falciparum were documented, and continuous surveillance will be needed to evaluate the progression of treatment failure incidence.

Clearance of parasites took more than 1 day in 75.8 percent of the subjects and more than 2 days in 17.6 percent of the subjects. Our results are similar to those recently reported in a study conducted in The Gambia in which 81 percent of the children treated with SP were still parasitemic on day 1 and 19.2 percent were parasitemic on day 2 (19).

We evaluated five mutations in P. falciparum DHFR and DHPS that have been reported to be associated with SP resistance. As reported in studies from other countries with low incidence of treatment failure (3), only three of these mutations were found in our study: the DHFR mutations at codons 108 and 51 and the DHPS mutation at codon 437. The mutation at DHFR codon 164, which was strongly associated with treatment failure in studies made in Bolivia and Peru (3, 20), was not found in this study.

We showed that falciparum infections clear more slowly if they carry parasites with DHFR mutations. Furthermore, the presence of both DHFR mutations at codons 108 and 51 was
more strongly associated with PCT than that of the mutation at codon 108 alone (relative hazard = 0.24 and 0.45, respectively), which supports the enhancing effect of multiple mutations in the in vivo response as has been described (3, 11, 12, 20). We did not find an association of PCT with the DHPS 437 mutation, although this mutation was always found in infections with doubly mutant DHFR, precluding an independent analysis of its contribution to PCT. This DHPS 437 mutation is common in epidemiologic surveys even in areas with sparing use of antifolates (3) and, like the DHFR serine to asparagine 108 mutation, while it may be a necessary first step toward a more fully resistant infection, by itself it is only a minor contributor to antifolate resistance (21). The impact on PCT of multiply mutant DHPS, which was not identified in this study, remains to be assessed.

We further evaluated the consistency of the association of DHFR mutations with PCT by considering potential confounding effects. In particular, parasitemia level at admission was significantly associated with PCT in our study and was also strongly associated with PCT in a study of predictors of mefloquine treatment in Thailand (22). Patients with higher parasitemia will have longer PCT, even if the infection is fully sensitive, because there are more parasites in the body to be cleared. Given that infections with \textit{P. falciparum} are often polyclonal (23), patients with higher parasitemias could also have a greater chance of carrying a resistant clone. However, we showed that the association between mutations and PCT remained significant after adjustment for parasitemia level at admission. We also adjusted by sulfadoxine and pyrimethamine concentrations 24 hours after therapy and found that mutations remained strongly associated with PCT.

The effect of poor nutritional condition on treatment response was also explored, and we found that only extremely low concentrations of plasma iron (diagnostic of iron deficiency anemia) were associated with a longer PCT. However, a multivariate analysis showed that this effect might be partially or totally explained by initial high parasitemia. Other nutritional indicators evaluated were not found to be associated with longer PCT. It is possible that evaluation of these indicators previous to the development of the malaria episode would give more precise estimates of nutritional condition, given that acute disease may alter some of these parameters. If this is true, misclassification of nutritional status might partly explain the lack of association.

Ideally, malaria treatment should result in gametocyte clearance to reduce transmission. This is particularly important in \textit{P. falciparum} because the gametocytes of this malaria species have a long lifespan of up to 24 days (24). Treatment

### Table 3. Determinants of parasite clearance time after sulfadoxine-pyrimethamine therapy in persons with uncomplicated falciparum malaria, Buenaventura, Colombia, 1999–2000

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative hazard</th>
<th>95% CI†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations at DHFR‡ codon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No mutation</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mutant 108 only</td>
<td>0.49</td>
<td>0.12, 1.99</td>
</tr>
<tr>
<td>Mutants 108 and 51</td>
<td>0.16</td>
<td>0.04, 0.65**</td>
</tr>
<tr>
<td>Parasitemia on day 0 (quartile)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (≤5,000)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2 (5,001–10,000)</td>
<td>0.32</td>
<td>0.10, 1.06*</td>
</tr>
<tr>
<td>3 (10,001–20,000)</td>
<td>0.36</td>
<td>0.10, 1.30</td>
</tr>
<tr>
<td>4 (&gt;20,000)</td>
<td>0.07</td>
<td>0.02, 0.27***</td>
</tr>
<tr>
<td>Pyrimethamine concentration 24 hours after treatment‡</td>
<td>2.02</td>
<td>0.77, 5.31*</td>
</tr>
</tbody>
</table>

* 0.05 < \( p < 0.15; \) ** 0.001 < \( p < 0.05; \) *** \( p < 0.001. \)
† CI, confidence interval; DHFR, dihydrofolate reductase.
‡ Drug concentration (µg/ml).

### Table 4. Odds ratios of occurrence of gametocytemia according to exposure to dihydrofolate reductase mutations at different days after treatment with sulfadoxine-pyrimethamine, Buenaventura, Colombia, 1999–2000†

<table>
<thead>
<tr>
<th>DHFR‡ codons</th>
<th>Day 3 Unadjusted OR‡</th>
<th>Adjusted OR</th>
<th>Day 7 Unadjusted OR</th>
<th>Adjusted OR</th>
<th>Day 14 Unadjusted OR</th>
<th>Adjusted OR</th>
<th>Day 21 Unadjusted OR</th>
<th>Adjusted OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mutation</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>108 mutant only</td>
<td>2.9</td>
<td>2.7</td>
<td>1.0</td>
<td>0.9</td>
<td>5.3*</td>
<td>5.1**</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>108 and 51 mutant</td>
<td>4.4</td>
<td>2.6</td>
<td>2.4</td>
<td>1.9</td>
<td>6.6*</td>
<td>4.4*</td>
<td>3.8*</td>
<td>3.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCT‡ (days)</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>3.9**</td>
<td>3.3*</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>7.9**</td>
<td>4.3</td>
<td>10.4**</td>
<td>3.8</td>
</tr>
</tbody>
</table>

* 0.05 < \( p < 0.10; \) **\( p < 0.05. \)
† Unadjusted and adjusted estimates by PCT.
‡ OR, odds ratio; DHFR, dihydrofolate reductase; PCT, parasite clearance time.
with SP has been reported to increase the proportion of gametocytes detected in blood smears of *P. falciparum*-infected patients (25). We found that infections with DHFR mutations have higher transmission potential as measured by gametocytemia. Transmission potential also increased with each additional day that subjects took to clear parasitemia after treatment with SP. Environmental factors (i.e., the addition of fresh erythrocytes) have been shown to influence the rate at which *P. falciparum* erythrocytic parasites differentiate to sexual stages (26), and “stress” is also thought to cause *P. falciparum* to commit to sexual development (27). Therefore, our findings indicating that gametocytemia is more frequent and of higher density in persons with longer PCT may be partially explained by the fact that PCT is a measure of the time that viable parasites are exposed to drug treatment and other stress factors.

We found that DHFR mutations were associated with longer PCT (in the range of 1–3 days) and the presence of gametocytes (from day 7 on). The most likely mechanism is that DHFR mutations, even if insufficient to permit the parasite to survive drug treatment, prolong parasite survival under drug pressure. This is reflected by the PCT and manifested by increased gametocytemia. Even though in our analysis DHFR mutations remained significantly associated with gametocytemia after adjustment for duration of PCT, this is probably due to the relative insensitivity of PCT as a measure of parasite survival, particularly when PCT is measured at 24 hourly intervals. In addition, failure to adjust for a factor that causes both longer PCT and increased gametocyte carriage would result in a residual association of the mutations and gametocytemia in the model we adjusted by PCT (28). While we adjusted for a number of likely factors, we did not directly measure or adjust for host immunity, which is a potential common cause of both longer PCT and gametocyte carriage. Age is frequently used as a surrogate for immunity in high-transmission settings such as Africa. We did not see an association between age and PCT or gametocyte carriage. Age is frequently used as a surrogate for immunity in high-transmission settings such as Africa. We did not directly measure or adjust for host immunity, which is a potential common cause of both longer PCT and gametocyte carriage. Age is likely to be a poor predictor of immunity, as evidenced by the wide age range of our symptomatic subjects.

Our data suggest that even in a setting where an antimalarial drug is highly efficacious and the parasite mutations that confer high-level resistance are absent, low-level drug resistance mutations may contribute to the potential for the transmission of *P. falciparum* and the spread of resistance. The prolonged parasite clearance associated with low-level DHFR mutations raises the possibility that increasing PCT could serve as a useful early warning sign of developing resistance before treatment failures become common. Our findings highlight the need to anticipate drug resistance before it arrives and to develop strategies for deterring the evolution and spread of resistance. Antimalarial drugs and drug combinations designed to eliminate both asexual and sexual parasites may deserve priority not only because they will reduce malaria transmission but also because they will reduce the spread of drug resistance in its earliest stages.

ACKNOWLEDGMENTS

Supported in part by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (ID–990223), by the STATEPI Group of the Johns Hopkins Bloomberg School of Public Health, by the Fundacion para la Educacion Superior, by the Valle State Secretary of Health in Colombia, and by US National Institute of Allergy and Infectious Diseases grant R01-AI-44824.

REFERENCES