ASSESSMENT OF THERAPEUTIC RESPONSE OF PLASMODIUM FALCIPARUM TO CHLOROQUINE AND SULFADOXINE-PYRIMETHAMINE IN AN AREA OF LOW MALARIA TRANSMISSION IN COLOMBIA

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Abstract. Although chloroquine (CQ) resistance was first reported in Colombia in 1961 and sulfadoxine-pyrimethamine (SP) resistance both in 1981, the frequency of treatment failures to these drugs in Colombia is unclear. A modified World Health Organization 14-day in vivo drug efficacy test for uncomplicated Plasmodium falciparum malaria in areas with intense malaria transmission was adapted to reflect the clinical and epidemiologic features of a low-intensity malaria transmission area in the Pacific Coast Region of Colombia. Patients ≥1 year of age with a parasite density ≥1,000 asexual parasites per microliter were enrolled in this study. Forty-four percent (24 of 54) of the CQ-treated patients were therapeutic failures, including 7 early treatment failures (ETFs) and 17 late treatment failures (LTFs). Four (6%) of 67 SP-treated patients were therapeutic failures (2 ETFs and 2 LTFs). Therapeutic failure in the CQ-treated group was associated with an age <15 years old (P < 0.01), but was not associated with initial parasite density, the presence of CQ or sulfa-containing drugs in urine, or a history of malaria. The high level of therapeutic failures to CQ detected in this study underscores the need and importance of drug efficacy evaluation in the development of a rational national antimalarial drug policy. The relatively low level of therapeutic failures to SP compared with other South American countries raises further questions regarding factors that might have prevented the rapid development of in vivo resistance to this drug combination.

Despite attempts at eradication, malaria remains a major public health problem in Colombia. During 1997, 180,910 malaria cases were reported to the Ministry of Health, of which 36% were caused by Plasmodium falciparum. The administrative departments of Chocó, Valle, Cauca, and Nariño on the Pacific coast accounted for 45% of the P. falciparum malaria cases diagnosed in 1997 in Colombia.

Since 1961, when chloroquine (CQ) resistance was first reported in Colombia,1 several studies have confirmed CQ and sulfadoxine-pyrimethamine (SP) resistance both in vivo and in vitro.2-4 In 1986, the National Malaria Control Program recommended a combination of CQ plus SP with a single-dose of primaquine (as a gametocidal agent) for the treatment of uncomplicated P. falciparum infection in areas without CQ resistance, and amodiaquine plus SP and primaquine for areas with CQ resistance. Although the national treatment policy differentiates areas with and without resistance to CQ, few studies have attempted to elucidate the distribution and magnitude of CQ resistance in Colombia.

The objective of our study was to determine the frequency of therapeutic failures to CQ and SP in patients with uncomplicated P. falciparum infection, using a 14-day in vivo test in an area of low intensity transmission in the northern Pacific coast of Colombia. At the same time, the study was used to train personnel from the Ministry of Health and regional malaria control programs from 3 Departments of Colombia in this methodology so that similar studies can be carried out in other areas and provide the basis for formulating a rational malaria treatment policy for Colombia.

METHODS

Protocol development. The protocol used for this study was a 14-day modification of the standard World Health Organization (WHO) 28-day in vivo test developed by the Centers for Disease Control and Prevention (Atlanta, GA) for malaria treatment efficacy testing in sub-Saharan Africa.5 A 14-day follow-up period was chosen because of a growing consensus among policy makers in Africa, Asia, and South America that national malaria treatment policy decisions should be based on clinical and/or parasitologic response during the first 14 days of follow-up. Shortening the follow-up period to 14 days also makes the study logistically simpler and less costly. Further changes were made to reflect the clinical and epidemiologic features of an area with low-intensity transmission in Colombia (<5%) including enrollment of patients ≥1 year of age with a parasite density ≥1,000 asexual parasites per microliter on a thick blood film, inclusion of patients both with and without fever at the time of enrollment, and treatment of all patients with parasitemia on or after day 7 independent of clinical symptoms (fever). No changes were made in the timing for follow-up visits or study procedures that are described in this report. This protocol is now recommended by WHO as its standard study methodology for assessing uncomplicated P. falciparum malaria treatment efficacy in areas of low to moderate intensity transmission.6

Study location. The municipality of Quibdó, the capital of the department of Chocó (Figure 1), is located on the northern Pacific coast region of Colombia. This city serves as a river port and commercial center of the region. In 1997, Quibdó had a population of 123,201 inhabitants, of whom 62% resided in the urban area. During that year, Quibdó reported 5,940 parasitologically confirmed cases of malaria, of which 66% were caused by P. falciparum. This annual infection rate of <5% is consistent with an area of low-intensity transmission. The Vector Borne Disease (ETV) program in Quibdó is responsible for the diagnosis and management of patients with malaria, and also provides support to municipalities in the surrounding areas with malaria control.
Enrollment procedures. Thick blood films were prepared for all patients who presented to the ETV program in Quibdó for evaluation. Patients with \textit{P. falciparum} infections were further evaluated to determine whether they satisfied the criteria of inclusion: age $\geq$ 1 year of age, pure \textit{P. falciparum} infections with $\geq$ 1,000 parasites/$\mu l$, the ability to return for follow-up visits during the subsequent 14 days, and agreement to participate in the study. Informed consent was obtained from the patient or the guardians of children. Patients were excluded from the study if they were pregnant, persistently vomited the study drug, had signs of severe malaria, or had an intercurrent illness that required an additional medication with antimalarial action, such as sulfonamides, tetracyclines, or clindamycin.\textsuperscript{7}

In agreement with WHO recommendations,\textsuperscript{6} a recent history of antimalarial drug use or the presence of CQ or sulfadiazine-containing drugs in the urine on enrollment (day 0) was not a reason for exclusion.\textsuperscript{8} Urine was tested for the presence of CQ using the Saker-Solomon test and for sulfadiazine-containing drugs using a test for field detection of sulfa-containing drugs in urine.\textsuperscript{9,10}

This study was approved by the Institutional Research Board of the Centro Internacional de Entrenamiento e Investigaciones Medicas, and was conducted in accordance with the Ministry of Health and the U.S. Public Health Service regulations governing the protection of human subjects in medical research.

Treatment and follow-up. The patients were randomly assigned to receive CQ or SP. The treatment was administered based on age, as is standard in Quibdó and many parts of the country. In the subsequent analysis data from those patients who weighed $< 60$ kg and received a $< 25$ mg/kg total dose of CQ or $< 25$ mg of sulfadoxine/kg were excluded from further analysis. Personnel from the research group administered all doses of the study drugs and the patients were observed for 30 min after dosing to ensure that they did not vomit the medication. In case of vomiting, the dose was repeated. Because this study intended to assess clinical efficacy in a real-life clinical setting, patients and providers were not blinded to the study medications administered to each patient. Potential biases from using this approach were limited because of the use of quantifiable study endpoints (parasite density and temperature). Patients were asked to return on days 1, 2, 3, 7, and 14 for follow-up for clinical assessment and blood slide examination. Hemoglobin levels were not measured.

Responses to treatment were classified in 2 ways: 1) parasitologic response graded as RIII, RII, early RI, and late RI/sensitive (S),\textsuperscript{10} and 2) therapeutic response according to the following classification, a) early treatment failure (ETF) defined by any one of the following: i) development of danger signs or severe malaria on days 1, 2, or 3 in the presence of parasitemia; ii) parasite density higher on day 2 than that on day 0; and iii) parasite density on day 3 $\geq$ 25% of that on day 0; b) late treatment failure (LTF) defined by any one of the following: i) development of danger signs or severe malaria\textsuperscript{7} after day 3 in the presence of parasitemia; ii) unscheduled return of the patient because of clinical deterioration in the presence of parasitemia; and iii) presence of parasitemia on any scheduled follow-up day on or after day 7; c) adequate clinical response: did not meet criteria for early or late treatment failure and clearance of parasitemia after day 3 through day 14.\textsuperscript{10,11} It should be noted that a proportion patients classified as an adequate clinical response might have been LTFs if they had been followed for 28 days.

Patients who failed treatment with CQ were treated with SP and those who failed SP were treated with quinine. If during follow-up patients were found to have gametocytemia, they were provided a mosquito net to limit the likelihood that those patients would be a source of ongoing transmission, and received a single dose of primaquine (0.75 mg/kg) at the end of follow-up.

Laboratory diagnostics and quality control. On enrollment and each follow-up day, 2 thick blood films were collected and stained with Field’s stain. Parasite density was calculated by dividing the number of asexual parasites by the actual number of leukocytes counted (approximately 300) and multiplying by 8,000 (the estimated number of leukocytes/$\mu l$ of blood). In order for a blood slide to be considered negative, at least 100 fields were examined. Two laboratory technicians with expertise in malaria microscopy read all blood slides. The second reader was unaware of patient’s identity and the result of the first reader. Slides with discordant results (positive/negative) were evaluated by a qualified third reader, as were positive slides with differences in the parasite density with coefficient of variations higher than 25% (calculated by quartiles).\textsuperscript{12} The measurement of the first or second reader that was nearest to that of the third reader was considered the true value and used for analysis.

Statistical analysis. Patients were included in the analysis if they received the correct dose of the study medicine, attended all follow-up visits, and did not take another antimalarial drug during the follow-up period. Epi-Info version 6.04 (Centers for Disease Control and Prevention) was used for generation of summary statistics and for comparison of therapeutic failures in each of the study arms. The chi-square test was used to determine difference of proportions and the t-test was used for the comparison of means.
**RESULTS**

Of the 728 patients who sought attention at the ETV program between March 1, 1997 and January 20, 1998 and who had blood slide-confirmed *P. falciparum* infections, 147 (20%) were enrolled in the study. Reasons for ineligibility were unavailability for follow-up (n = 376), parasite density <1,000 asexual parasites per microliter (n = 174), persistent vomiting (n = 7), other *P. falciparum* infection (n = 3), <1 year of age (n = 2), severe malaria (n = 3), and more than one reason (n = 16). Among the 147 patients included in the study, 69 were randomized to CQ and 78 to receive SP. Twenty-six patients were excluded from analysis because they took another antimalarial drug (4 in the CQ and 1 in the SP group), did not complete the follow-up (5 in the CQ and 9 in the SP group), the patient’s weight was not available (n = 1), or the patient had received an underdosage of CQ or SP (5 in the CQ and 1 in the SP group). All patients who received less than 25 mg/kg of CQ met criteria for therapeutic failure during follow-up.

Demographic characteristics, geometric mean parasite density at enrollment, and history of previous malaria were comparable between evaluable patients in both groups, but a higher proportion of patients randomized to CQ were positive for CQ on urine testing (*P < 0.05*) (Table 1).

Of the 54 evaluable patients treated with CQ, 7 (13%) were classified as RIII, 10 (18%) as RII, 7 (13%) as early RI, and 30 (56%) as late RI/S. Sixty-seven SP-treated patients were evaluable, of which 2 (3%) were RIII, 1 (1.5%) was RII, and 63 (94%) were late RI/S. Therapeutic responses according to the most recent who classification are shown in Table 2.1

When patients who responded to CQ were compared with those who failed CQ, a stratified analysis showed that patients <15 years of age were more likely to be classified as therapeutic failures (combined ETFs and LTFs) (*P < 0.01*). Those 15–44 years of age were more likely to demonstrate an adequate clinical response (*P < 0.05*) (Table 3). The geometric mean parasite density on enrollment, the presence of antimalarial drugs in urine, and a history of malaria did not differ between therapeutic failures and responders (Table 3).

Of the 102 eligible patients who underwent urine testing, 28 (27%) and 9 (9%) of patients, respectively, tested positive for the presence of CQ and sulfa-containing drugs in the urine; of these 6% (n = 2) had used both. It should be noted that 17% and 0%, respectively, of those who tested positive reported at the initial interview that they had taken these antimalarial drugs.

**DISCUSSION**

Although several previous malaria treatment studies in Colombia have documented resistance of *P. falciparum* to CQ and SP, only one report has been published in the past decade.13 This lack of information on the distribution and magnitude of antimalarial drug resistance in Colombia has resulted in confusion and prevented the formulation of an adequate treatment policy. Furthermore, previous studies have not used standardized methods, thereby making their results difficult to compare. Our study is the first step in assessing the current therapeutic efficacy of CQ and SP in representative malaria-endemic regions in Colombia.

In the municipality of Quibd, we observed a high proportion of therapeutic failures to CQ (44%). The results of our study are consistent with those of investigations carried out in Tumaco, Nariño (southern Pacific coast) in 1988 and Buenaventura, Valle (middle Pacific coast) in 1995 (Barrera L and others, unpublished data), which found that 40.5% and 50%, respectively, of patients treated with CQ were thera-
Comparison between patients classified as therapeutic failures with those who responded to treatment with chloroquine (CQ)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Therapeutic failure n = 24 (%)</th>
<th>Therapeutic response n = 30 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean parasite density on enrollment (range)</td>
<td>5,284/µl (1,000–35,364)</td>
<td>4,008/µl (1,000–31,771)</td>
</tr>
<tr>
<td>Median age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–14</td>
<td>12 (8)</td>
<td></td>
</tr>
<tr>
<td>15–44</td>
<td>14 (58)</td>
<td>23 (77)</td>
</tr>
<tr>
<td>45–59</td>
<td>7 (29)</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>2 (8)</td>
<td>0</td>
</tr>
<tr>
<td>History of malaria in the last 6 months</td>
<td>2/23 (9)</td>
<td>4/29 (14)</td>
</tr>
<tr>
<td>Presence of CQ in urine</td>
<td>5/17‡ (29)</td>
<td>10/23 (43)</td>
</tr>
<tr>
<td>Presence of sulfa drugs in urine</td>
<td>1/17‡ (6)</td>
<td>5/23 (21)</td>
</tr>
</tbody>
</table>

* P < 0.01
‡ P < 0.05
‡ Number of patients with the characteristic divided by the number of patients whose data were available.

The frequency of therapeutic failures is well above the generally accepted level (25%) of treatment failure considered by WHO to warrant a change in the treatment policy. This critical value of 25% treatment failures in a 14-day study is based on the consensus opinion of researchers, malaria program managers, and policy makers, and on cost-effectiveness studies that favor changing the first-line drug above this level. Other factors should be taken into account when modifying malaria treatment policy, including the efficacy, toxicity, and cost of possible replacement drugs, the cost and effort of implementing a change in policy, and community and health care worker perceptions and expectations of treatment.

The frequency of SP treatment failures (6%) identified in Quibdó is similar to the 10% failure rate described by Soto and others in soldiers in the neighboring Department of Antioquia, but differs from a previous study in the same region in 1985 in which 25% of the patients failed treatment with SP. Resistance to SP has also been reported in 9 patients from the Amazonia/Orinoquia region of Colombia, although systematic efficacy testing has not yet been carried out in this area. Despite the fact that SP has been in use in this area as a first-line treatment for more than 10 years, the proportion of treatment failures to SP found in Quibdó was lower than expected. In Southeast Asia and other parts of South America, resistance to SP has developed much more rapidly. A high frequency of dihydrofolate reductase Asn-108 mutations has been observed in samples taken from patients enrolled in our study. This may be an indicator of the accumulation of other mutations that are associated with the development of in vivo resistance, suggesting that conditions exist in the area for the future emergence of clinical resistance to SP. However, the national policy that recommends the use of a multiple drug regimen for treatment of uncomplicated malaria (CQ, SP, and primaquine) may explain, in part, why SP resistance has developed more slowly in Quibdó, particularly when one considers the high prevalence of SP resistance reported in neighboring Brazil and the Peruvian Amazon region, and where SP monotherapy has been used. Although there may be other explanations for the low levels of SP resistance we identified, the potential role that multiple drug therapy has played in delaying the emergence of resistance to SP warrants further investigation. Evaluation of the efficacy of SP in the Amazon Region of Colombia, where combination therapy is used and transmission intensity is similar to areas of Brazil and Peru where high-level SP resistance has been reported, may provide important evidence to support the theory that the use of multiple drug treatment has slowed the development of SP resistance.

Our experience has further demonstrated that the study methodology, which was initially designed for testing in areas of intense transmission in Africa, can be easily modified for use in areas with low-intensity malaria transmission. Only inclusion criteria and clinical definitions for therapeutic failure were modified to reflect the local epidemiology of malaria in this region. Unlike Africa, where the diagnosis of malaria is commonly based solely on clinical findings, the diagnosis of malaria in Colombia is almost always based on identification of parasites on a blood slide. Similarly, treatment failures are frequently identified when routine follow-up blood slides demonstrate persistent or recurrent parasitemia. Given these distinctions in the use of microscopy in the management of persons with malaria, it is logical to emphasize parasitologic criteria for enrollment and designation of treatment failure over clinical findings in our study methodology. The WHO and the Pan American Health Organization now recommend an almost identical methodology for assessment of in vivo malaria treatment efficacy in areas or low to moderate-intensity transmission.

National and local antimalarial treatment policy recommendations should be guided by in vivo drug efficacy testing in representative areas using a standardized methodology. To have comparable data that can rationally inform national drug policy, these should use the same inclusion criteria, procedures to measure parasite density, follow-up schedule, and outcome measures. Training was concomitantly achieved during the conduct of this study via hands-on experience. This strategy has facilitated the development of efficacy evaluations in other representative areas of malaria transmission in Colombia. Because of the high levels of clinical resistance to CQ identified in this study, the Departamento Administrativo de Salud del Chocó has changed from CQ to amodiaquine, plus SP and primaquine, for treatment of uncomplicated P. falciparum infection in Quibdó. Ongoing monitoring of the recently modified therapy using a standardized methodology and rigorous quality control is essential to verify as well as maintain its efficacy.
The association between an age of 1–14 years and therapeutic failure to CQ suggests that young age may predispose to therapeutic failure in areas of low-intensity transmission. A high frequency of therapeutic failure in response to CQ among children was also reported by Comer and others in 3 indigenous communities in north Chocó in 1968. In their study, the dosage of chloroquine was based on a weight group table. They enrolled 57 patients, observing 24 (42%) failures to clear the parasites or recrudescence by day 28. Of these 24 patients, 22 (92%) were ≤10 years of age and 15 of them received more than 25 mg/kg of chloroquine.

In our study, we used the age-based scale to provide CQ treatment, as recommended by the national malaria control program. When the CQ dosage was calculated using the actual patient weight; however, the age-based table often underestimated the correct dose by weight. Thus, the use of the age-based scale for the treatment of children should be reconsidered to ensure that young children receive an appropriate treatment dose. Nevertheless, because all patients (weight <60 kg) who received less than 25 mg/kg were excluded from the analysis, the rates of therapeutic failures observed in this study cannot be explained by inadequate CQ dosing.

Several factors may contribute to this association between age and clinical response to treatment, which has been described in similar studies in Africa, including less protective immunity acquired from previous infections in children, difficulty in the ingesting of the drug, and inaccuracy of the immunity acquired from previous infections in children, difficulty in the ingesting of the drug, and inaccuracy of the dosage when fragments of tablets are used. Age-associated variations in the pharmacokinetics of CQ does not appear to affect treatment outcome because CQ is well absorbed both in children and adults.

This study underscores the vulnerability of children in the clinical and treatment response and the importance of including them in efficacy evaluations. The higher frequency of treatment failure in children raises the question of whether acquired immunity or intrinsic differences in susceptibility between adults and children may obscure the early detection of drug resistance in adult population.

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