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EFFICACY OF THREE CHLOROQUINE–PRIMAQUINE REGIMENS FOR TREATMENT OF *PLASMODIUM VIVAX* MALARIA IN COLOMBIA

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Abstract. *Plasmodium vivax* malaria is an important cause of morbidity in Central and South America. In Colombia, this is the most prevalent malaria infection, representing 75% of the reported cases. To define the efficacy of the chloroquine and primaquine regimen to eliminate hypnozoites and prevent relapses, we conducted a random controlled clinical trial of three primaquine regimens in an open-label study. We evaluated the anti-relapse efficacy of total primaquine doses of 45, 105, and 210 mg administered at a dosage of 15 mg/day in 210 adults with *P. vivax* infection from the northwestern region of Colombia. Cure rates for blood-stage *P. vivax* malaria by day 28 of follow-up were 100% in all groups. Post-treatment reappearance of parasitemia during the six months of follow-up was 45%, 36.6% and 17.6%, respectively, for each group. When compared with other groups, administration of 210 mg was a significant protection factor for reappearance of parasitemia in a malaria-endemic area.

INTRODUCTION

Malaria causes an acute, debilitating febrile syndrome that causes death in 1.5–2.7 million of approximately 500 million persons infected annually.[1] Of the four protozoan species of the genus *Plasmodium* that cause infection in humans, *P. falciparum* and *P. vivax* are responsible for most cases of malaria. *Plasmodium vivax* causes malaria in approximately 80 million people annually, predominantly in Asia, the Western Pacific, the Middle East, and the Americas.[2]

In Colombia, *P. vivax* is responsible for 75% of the cases of malaria in the country, which results in confirmed infection in more than 100,000 inhabitants per year.[3] Although chloroquine (CQ)–resistant *P. vivax* has been reported in Colombia,[4] almost all acute *P. vivax* infections can still be treated with CQ successfully in this country.[5] To prevent relapses of *P. vivax* arising from persistent liver stages (hypnozoites), radical treatment with primaquine (PQ) is required. The standard dose of PQ (0.25 mg/kg of base, 15 mg/day for 14 days for an adult) is recommended by the World Health Organization,[6] as well as by the Ministry of Health of Colombia. Primaquine prevents relapse, but confusion surrounds its use. Among the several widely used regimens, none has been adequately evaluated. In some regions where malaria is endemic, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common trait.[7] Therefore, administration of the full 14-day course of PQ to G6PD-deficient persons is contraindicated because of the risk of severe hemolysis.[8] To overcome this problem, some governments have adopted a truncated five-day course of PQ for *P. vivax* malaria because this reduces the risk of hemolysis to negligible levels.[9]

Researchers from India and Pakistan confirmed that a five-day course PQ (15 mg/day) was ineffective in preventing recurrences of *P. vivax* malaria.[9] In Brazil, administration of CQ at a standard dose and a five-day course of PQ (30 mg/day) was effective only in 80% of patients in preventing recurrence of *P. vivax* malaria.[10] Moreover, Bunnag and others reported that in Thailand a PQ dose of 22.5 mg/day for 14 days was more effective in preventing prevent *P. vivax* malaria recurrences than the standard dose of 15 mg/day for 14 days.[11] Several investigators then proposed (reviewed by Baird and Rieckmann[8]) that it is the total dose of PQ administered rather than the duration of treatment that determines the efficacy of PQ. However, an abbreviated regimen of PQ has been proposed by Bergonzoli and Rivers Cuadra to be equally effective and is widely used without proven clinical efficacy.[12] In Colombia, recent studies confirmed the clinical efficacy of CQ in eradicating asexual forms of *P. vivax*,[5,13] but no studies have been performed in this country that evaluated the efficacy of PQ in preventing relapses.

To determine the rate of failures to PQ, we designed a prospective clinical trial based on three regimens in an open-label comparison. Patients were followed-up for six months in an area of *P. vivax* transmission located in northwestern Colombia.

MATERIALS AND METHODS

We conducted a non-blind random clinical and controlled trial without placebo. A total of 210 patients with *P. vivax* malaria were recruited and allocated a treatment using an Excel® (Microsoft, Redmond, WA) function. Three treatment groups were used: group I received 45 mg of PQ (26.3-mg tablets with 15 mg of PQ base; Sanofi-Synthelabo, Paris, France) over a three-day period, group II received 105 mg of PQ over a seven-day period, and group III received 210 mg of PQ over a 1 14-day period. All patients were administered 600 mg of CQ (Sanofi-Synthelabo), 250-mg tablets with 150 mg of CQ base, on day 0 and 450 mg on days 1 and 2 (total dose = 1,500 mg) after diagnosis. All treatments were supervised. During the first 28 days of treatment, response to CQ plus PQ was assessed; thereafter, the frequency of recurrences was evaluated until six months after treatment.

Study area. The study was carried out in two malaria endemic municipalities of Colombia: Turbo (8°5′42″N, 76°44′123″W) and El Bagre (7°35′25″N, 74°48′27″W) (Figure 1). These regions are inhabited mainly by people of African origin although mixing with indigenous and Spanish descendants is observed. The economy of El Bagre is based on gold mining. Turbo is a banana production area. Both municipali-

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FIGURE 1. Geographic location of the study regions in Colombia.

ties have high migration rates and malaria transmission is perennial and unstable with mean annual parasites indexes during 2001–2003 of 19.65 in Turbo and 37.35 in El Bagre.[3]

Sample size. A sample was selected by using the Lwanga and Lameshow method[14] to test the differences in proportion between two independent populations (5% alpha error and 20% beta error) with a minimum 15% difference in therapeutic efficacy. This resulted in a sample size of 58 patients per each group. This number was increased to 70 to compensate for dropouts; therefore, the total number of individuals was 210.

Patient enrollment and follow-up. This study included patients attending the local malaria clinics with acute symptomatic *P. vivax* malaria and a positive thick blood smear between September 2003 and September 2004. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universidad de Antioquia (Medellín, Colombia). Each participant gave fully informed consent. Children were not included because of ethical constrains. The inclusion criteria for the study were an age ≥ 15 years, *P. vivax* parasitemia of ≥ 1,000 asexual forms/μL, permanent residency in the municipalities of the study, willingness to give informed consent, a normal G6PD screening test result, and an agreement to attend follow-up for six months. Patients who were pregnant were excluded, as were those with associated infectious diseases, a history of anti-malarial intake during the previous two weeks, diarrhea or vomiting (> 5 episodes in 24 hours), hypersensitivity to anti-malarials or intake of any anti-malarial different from those provided by the researchers, travel to a different municipality, severe undernourishment, symptoms or signs of severe malaria (according to the World Health Organization),[15] and consent withdrawal.

After clinical evaluation and examination of thick and thin blood smears to establish the diagnosis, patients were randomly assigned, in an open fashion, to one of the three treatment groups. Treatments were supervised during the 14 days of treatment. Patients were followed-up by active surveillance at the clinic or their homes for six months, and any further episodes of malaria were confirmed by microscopy and given the same course of treatment (for up to two more times) as at enrollment. If parasitemia reappeared a third time, PQ (plus a standard dose of CQ), 15 mg/day for 28 days, was given. Any *P. vivax* parasitemia observed after day 28 in patients with adequate treatment response was defined as a recurrence. Therefore, both relapse and re-infection were included in this definition.

Monitoring of parasitemia. A parasite count was obtained using Field-stained thick blood films and defined as the number of asexual parasites per 200 white blood cells. Capillary blood was taken before treatment and then every 24 hours until negative; thereafter, smears were examined daily for the first three days, then on days 28, 60, 120, and 180 after treatment. The thick blood films were considered negative if no parasites were seen in 200 oil-immersion fields.

Statistical analysis. Data were analyzed using SPSS version 10.0 (SPSS Inc., Chicago IL). Continuous variables are summarized as means and SDs and compared by one-way analysis of variance. The chi-square test was used to compare dichotomous variables. Recurrences were compared with relative risk, absolute risk reduction, and number needed to treat. All patients were included in analysis of efficacy (to time of recurrence or loss to follow-up) and all outcomes were included for analysis, regardless of compliance with dosing regimens (intent-to-treat). The survival of recurrences was made by Kaplan-Meyer analysis to determine the average time of the

TABLE 1
Baseline characteristics of study patients with *Plasmodium vivax* in three evaluated groups, September 2003–September 2004*

Variables	45 mg PQ n = 71	105 mg PQ n = 71	210 mg PQ n = 68	Total N = 210	*P*
Age (years)	31.0 ± 13.8	30.0 ± 13.4	29.4 ± 11.1	30.1 ± 12.8	0.758
Parasitemia (μL)	6,174 ± 6,538	5,423 ± 5,075	6,161 ± 7,297	5,955 ± 6.344	0.690
Weight (kg)	62.5 ± 9.8	61.5 ± 8.4	60.4 ± 9.0	61.5 ± 9.1	0.416
Days with symptoms prior to baseline	4.7 ± 3.2	5.0 ± 4.8	4.8 ± 3.0	4.8 ± 3.7	0.873
Previous history of malaria	27 (37%)	28 (39%)	23 (34%)	78 (37%)	0.642
Number of previous malaria episodes	2.6 ± 2.4	2.3 ± 1.6	1.8 ± 1.3	2.2 ± 1.9	0.389
% male	49 (57%)	53 (75%)	40 (59%)	142 (67%)	0.140
Mestizo ethnia	67 (92%)	65 (91%)	63 (93%)	165 (92%)	0.957

* Values are mean ± SD unless otherwise indicated.
PQ = primaquine.

TABLE 2
Frequency and percentage of relapses and lost to follow-up, and summary of recurrences survival in days*

	Group 1 45 mg PQ	Group 2 105 mg PQ	Group 3 210 mg PQ	Total
Number of cases	71	71	68	210
Recurrences	32 (45.1%)	26 (36.6%)	12 (17.6%)	70 (33.3%)
Adequate treatment response	33 46.5%)	36 (50.7%)	52 (76.5%)	121 (57.6%)
Lost	6 (8.5%)	9 (12.7%)	4 (5.9%)	19 (9%)
Censured cases	39 54.9%)	45 (63.4%)	56 (82.3%)	140
Mean survival	136	143	168	149
(95% CI)	(124, 149)	(130, 156)	(161, 175)	(142, 156)

* PQ = primaquine; CI = confidence interval; censored cases = no. of patients lost to follow-up or who finished the study without recurrence. Log-rank *P* value = 0.0008.

recurrences in each regimen of treatment. Log-rank and Breslov analyses was used to compare the recurrence curves. For the purpose of survival analyses, if an individual did not have a recurrence during the five months of follow-up or if an individual was lost to follow-up before experiencing any recurrence of *P. vivax* malaria, this individual was censored at the time of his or her last follow-up visit with a malaria smear examination. All reported confidence intervals were 95% and all *P* values were two-sided. The Kolmogorov-Smirnov test was used to test for normality of continuous variables. The Kruskal-Wallis test was applied to variables lacking a normal distribution. A level of significance of 5% was always assumed.

RESULTS

Patients. Of the 210 patients studied, 138 were recruited in Turbo and 72 in El Bagre. Seventy-one patients were allocated to group I, 71 to group II, and 68 to group III. Between days 28 and 180 of the follow-up period, 19 patients were lost; these were equally distributed among the three groups of study and had no effect on the results observed. Demographic, clinical, and pretreatment laboratory data are shown in Table 1. There were no significant differences between the treatment groups ($P > 0.05$). Parasite clearance was confirmed in 97% of the patients by day three after treatment and in 100% by day 28 after treatment.

Treatment and follow-up. Although relapse and new infections could not be distinguished by microscopy, over the six months of follow-up, group III appeared protected against further episodes, as shown by a statistically significant reduced rate of recurrence of parasitemia when compared with groups I and II (Table 2). Overall, 33% (n = 70) individuals had a subsequent attack of *P. vivax* malaria; of these, 16 patients had a second episode and three had a third episode. Of those subjects who were administered the three-day course of PQ (15 mg/day, total dose = 45 mg), 19 (27%) of 71 had one recurrence, 12 (17%) of 71 had two recurrences, and 1 (1.4%) of 71 had three recurrences. In subjects administered the seven-day course of PQ (total dose = 105 mg), 21 (29.5%) of 71 had one recurrence and 3 (4.2%) of 71 had two recurrences, and 2 (2.8%) of 71 had three recurrences. In the group administered the 14-day course (total dose = 210 mg), 11 (16.2%) of 68 had one recurrence and 1 (1.5%) of 68 had two recurrences. No additional recurrences were observed in this group.

The mean ± SD number of recurrences was 0.71 ± 0.82 (total dose = 45 mg) in group I, 0.56 ± 0.74 (total dose = 105 mg) in group II, and 0.2 ± 0.44 (total dose = 210 mg) in group III. The mean ± SD number of days between recruitment and recrudescence was 91 ± 38 days. However, this was significantly different in groups I and II (88 ± 37 and 85 ± 37) from that in group III (92 ± 38). The survival risk of recurrence is summarized in Figure 2 and Table 2. The higher-dose regimen of PQ (i.e., group III) had a lower risk of recurrence when compared with the other groups.

Results obtained after statistical analysis per protocol are shown in Table 3. The number of lost patients during the follow-up period had no effect on the final results, as confirmed after per protocol, intend-to-treat, and worst-case scenario analysis. Furthermore, each analysis confirmed that a dose of 210 mg of PQ over a 14-day period constituted a protective factor against recurrences of *P. vivax* malaria within 180 days.

DISCUSSION

Primaquine has been used for preventing relapse of malaria caused by hypnozoites in the liver at a standard dose of 15 mg/day for 14 days. The question addressed by the present study was the efficacy of shorter treatment regimens of PQ in two malaria-endemic regions of Colombia. Random alloca-

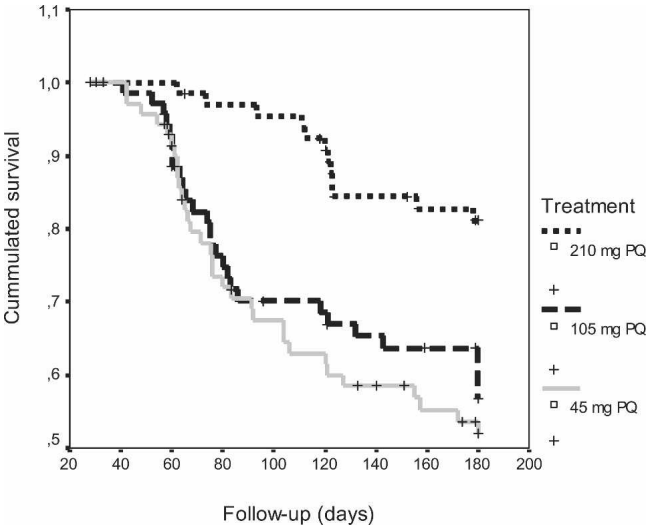

FIGURE 2. Distribution of survival according to the treatment. Kaplan-Meier survival curve of time from treatment with three chloroquine and primaquine (CQ + PQ) regimens of *Plasmodium vivax* infection to recurrence with *P. vivax* malaria in Colombian subjects.

TABLE 3
Analysis per protocol of three choloroquine and primaquine regimens in *Plasmodium vivax* infection in Colombian subjects*

Per protocol												
	Recurrences				Recurrences				Recurrences		
Dose	Yes	No	Total	Dose	Yes	No	Total	Dose	Yes	No	Total
210 mg	12	52	64	210 mg	12	52	64	105 mg	26	36	62
45 mg	32	33	65	105 mg	26	36	62	45 mg	32	33	65
RR = 0.38 (0.21, 0.67)				RR = 0.44 (0.24, 0.80)				RR = 0.85 (0.58, 1.25)			
ARR = 0.30				ARR = 0.23				ARR = 0.07			
NNT = 4 (3–7)				NNT = 5 (3–12)				NNT = 14 (5–11)			

* RR = relative risk; ARR = absolute risk reduction; NNT = no. needed to treat. Value in parentheses are 95% confidence intervals.

tion of individuals to one of three different regimens guaranteed similar baseline conditions for all groups. Cure rates for blood-stage *P. vivax* malaria by day 28 of follow-up were 100% in all groups. Post-treatment reappearance of parasitemia during six months of follow-up was 45%, 36.6%, and 17.6% for group I, group II, and group II, respectively.

All patients studied had an adequate treatment response to the combination of CQ and PQ, which confirmed previous reports in the region,[5,11] but this response differed from those of several reports from South America.[4,16–18] Taking into account the criteria reported by Ruebush and others[18] to assess treatment response to CQ (i.e., administration of supervised treatment with 1,500 mg of CQ, follow-up for 28 days, and measurement of plasma levels of CQ > 100 ng/mL), only the studies performed by these investigators and by Phillips and others[17] fulfill these criteria. Since we confirmed adequate treatment response in all patients in our study, assessment of plasma levels of CQ were not needed.

Post-treatment reappearance of parasitemia may arise from re-infection, relapse, or recrudescence. Based on the difference in the number of individuals who showed reappearance of parasitemia between group I and group III (n = 32 versus n = 12), the estimated risk reduction was 30% of the cases in group I. Thus, the recurrence rate may be as high as 17% (12 of 68) based on the standard PQ regimen (15 mg for 14 days) and the shorter three-day and seven-day regimens were less effective.

Baird and Hoffman[19] have reported that in tropical regions the duration between primary parasitemia and relapses in *P. vivax* is shorter, between two and four months, than in subtropical regions. We observed a mean ± SD time of recurrence of parasitemia of 92 ± 38 days, which is within the limits of relapse for tropical strains reported by these investigators. Furthermore, our studies may confirm previous reports of the multiplicity of relapses seen in *P. vivax* from tropical areas,[19] since two and up to three recurrences were observed in some patients.

Several investigators have reported that sub-therapeutic doses of PQ are associated with *P. vivax* relapses.[10,20,21] In our study, the mean weight was comparable in patients with or without recurrences in any group; therefore, the patient's weight had no effect on our observations.

Bergonzoli and Rivers Caudra[12] reported the efficacy of short CQ plus PQ regimens to prevent recurrences for up to six months in patients with *P. vivax* malaria in Costa Rica and Nicaragua. This observation could not be confirmed in the present study. Possible explanations for this might be either the circulation of different *P. vivax* strains in these regions and/or different degrees of endemicity between the areas studied.

Survival analysis of recurrence confirmed that standard treatment with PQ (15mg/day for 14 days) is significantly more effective than shorter regimens in preventing a recurrence. This regimen reduced the risk of recurrences (absolute risk reduction) between 23% and 30% (Table 3) when compared with patients administered the same dose for seven days or three days, respectively.

The per protocol, intend-to-treat, and worst-case scenario analyses confirmed that the number of losses during the follow-up period had no effect on the results obtained. Moreover, all three analyses confirmed that a dose of 210 mg of PQ was protective against recurrences during at least 180 days. Thus, administration of 210 mg of PQ for 14 days in patients with *P. vivax* malaria can reduce the risk of recurrences within 180 days.

The per-protocol analysis showed that treatment of 4–5 patients is required for absence of recurrences in at least 1 patient in < 180 days in the group administered 210 mg of PQ for 14 days. In the group administered 105 mg of PQ, the same result can be achieved after treatment of 14 patients.

Our findings suggest that a lack of efficacy of PQ against hypnozoites might be present in Colombia. Considering that the 14-day regimen did not prevent recurrences and that some might be caused by relapses, we suggest that testing higher daily and total doses (i.e., 30 mg/day for 14 days) of PQ, after assessment of G6PD status in patients, can be useful to elucidate these recurrences. Adequate adherence to treatment in field conditions is essential; therefore, shorter regimens (< 14 days) of at least the 210-mg total dose would be desirable.

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