Efficacy of Three Different Regimens of Primaquine for the Prevention of Relapses of *Plasmodium vivax* Malaria in the Amazon Basin of Peru

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Abstract. We evaluated the efficacy of three primaquine (PQ) regimes to prevent relapses with *Plasmodium vivax* through an open-label randomized trial in Loreto, Peru. Vivax mono-infections were treated with chloroquine for 3 days and PQ in three different regimes: 0.5 mg/kg per day for 5 days (150 mg total), 0.5 mg/kg per day for 7 days (210 mg total), or 0.25 mg/kg per day for 14 days (210 mg total). Biweekly fever assessments and bimonthly thick smears were taken for 210 days. Recurrences after 35 days were considered relapses. One hundred eighty cases were enrolled in each group; 90% of cases completed follow-up. There were no group-related differences in age, sex, or parasitemia. Relapse rates were similar in the 7- and 14-day regimes (16/156 = 10.3% and 22/162 = 13.6%, $P = 0.361$) and higher in the 5-day group (48/169 = 28.4%, $P < 0.001$ and $P = 0.001$, respectively). The 7-day PQ regimen used in Peru is as efficacious as the recommended 14-day regimen and superior to 5 treatment days.

INTRODUCTION

Plasmodium vivax malaria is an important public health problem in the Americas, causing 70% of the reported malaria cases in the region.¹ Chloroquine (CQ), a blood schizonticide, is the first line of treatment of vivax malaria in most parts of the world where resistance has not developed. In addition, primaquine (PQ) is used to kill the hypnozoites present in the liver, which are responsible for *P. vivax* relapses.² PQ also eliminates gametocytes in the blood, preventing transmission to mosquitoes.³ There is evidence that PQ synergizes with CQ to kill the asexual blood stage of *P. falciparum* and *P. vivax*,⁴ despite the minimal effect of PQ by itself against asexual blood parasites.⁵

After treatment, a recurrence of *P. vivax* in the bloodstream can be classified as recrudescence, relapse, or reinfection. Recrudescence is the reappearance of the blood-stage parasites originating from subpatent trophozoites in the blood that survive treatment because of either inadequate dosing or resistance of the parasites to the therapy. Relapse, however, is the recurrence of blood-stage parasites originating from latent hypnozoites in the liver.⁶ Relapses can occur anytime after 17 days after treatment, but if CQ is the blood schizonticide administered and the parasites are susceptible, the long half-life of CQ will prevent the reappearance of parasitemia before 35 days.² Finally, reinfection occurs when the patient is infected with a new strain of malaria by a separate mosquito bite any time after the initial infection. Both recrudescence and relapse indicate failure of the drugs to kill the parasites because of either resistance or suboptimal dosing.

The efficacy of PQ treatment regimens for the radical cure of *P. vivax* malaria depends more on the total dose of PQ administered than on the duration of the regimen.^{7,8} It is particularly important in the Americas, where the susceptibility of *P. vivax* to PQ is greater than the strains in southeast Asia and the Pacific.^{9,10} The standard PQ regimen recommended by the World Health Organization (WHO) for the Americas to pre-

vent relapse is 0.25 mg/kg per day for 14 days,¹¹ but this regimen introduces a substantial risk of limited adherence, because PQ is given mainly after patients have become afebrile and asymptomatic. Therefore, to maintain high adherence and prevent relapses, in 1999, the malaria control programs of Peru and other countries in the Americas adopted a 7-day regimen at 0.5 mg/kg per day, an approach with a total dose equivalent to the 14-day WHO recommendation.¹²

The evaluation of the efficacy of shorter courses of PQ to prevent relapses is critical for supporting existing policies in the Americas and determining potential refinements. Shorter 7-day PQ regimes had not been tested in Peru before the policy change, and 7-day or shorter regimes had shown mixed results in other South American countries.^{13–15} Also, the greater susceptibility of *P. vivax* strains to PQ in the Americas compared with strains from southeast Asia and the Pacific¹⁰ could offer additional room to benefit from shorter regimes with increased adherence. Therefore, we decided to compare the efficacy to prevent relapses of the WHO standard 14-day PQ regimen versus shortened regimes of 7 and 5 days that have been applied throughout the Americas.

MATERIALS AND METHODS

We compared the efficacy of three PQ dosing regimes (5, 7, and 14 days) to prevent relapses in days 35–210 post-treatment initiation. All recurrences in that period were considered relapses because of the low endemicity of *P. vivax* malaria in the Peruvian Amazon Basin and the limitations in accurately distinguishing reinfection from actual relapse molecularly. This study was conducted in the periphery of the city of Iquitos, which is located on the river bank of the Amazon River (Figure 1); it is the largest city in the Peruvian rainforest, with a population of approximately 400,000 inhabitants. In this region, malaria is hypendemic and seasonal, and it has unstable transmission, which makes the risk of reinfection less likely, although not negligible. The peak transmission usually takes place between February and April, and the main vector is *Anopheles darlingii*.¹⁶ This study was carried out at the Padre Cocha and the San Juan Health Centers between March of 2006 and August of 2008 and the

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FIGURE 1. Study sites in the Peruvian Amazon region.

Santa Clara Health Center between June of 2007 and August of 2008. The protocol was approved by the Institutional Review Board of the US Naval Medical Research Center (Protocol NMRCD.2005.0005) and the National Institutes of Health of Peru (Protocol 009-2004) in compliance with all federal regulations governing the protection of human subjects. The study was recorded in the Peruvian registry of clinical trials (<http://www.ins.gob.pe/registroec/recuperarECPB.asp?numEC=068-04&val=&NroPag=1&flg=0>; register 068-04, ID RA904) but not in an international registry. All subjects provided written informed consent, and all children 8–17 years old were asked to provide verbal assent to participate in the study.

PATIENTS AND PROCEDURES

This study used a revised version of the methods recommended by the WHO and the Pan American Health Organization (PAHO) for the assessment of antimalarial efficacy,¹⁷ because to date, there are no standard approaches to evaluate the efficacy of antimalarials to prevent *P. vivax* relapses.

Patients with a microscopy-confirmed diagnosis of mono-infection with *P. vivax* between 250 and 100,000 asexual parasites/ μ L (determined by microscopic examination of thick and thin peripheral blood smears), fever defined as axillary temperature $\geq 37.5^{\circ}\text{C}$ and/or history of fever, and ≥ 1 year old were enrolled. Pregnant and lactating women, patients with chronic illnesses, patients with symptoms of severe malaria, and patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency were excluded (Trinity Biotech, St. Louis, MO). After a consenting case of vivax malaria was identified in one of three health centers, a blood sample anticoagulated with ethylene diamine tetraacetic acid (EDTA) was taken to rule out G6PD deficiency and perform genotyping of the parasites detected at baseline.

The patients were assigned by a computer-generated block randomization table to one of three PQ treatment groups: (1) 0.5 mg/kg per day for 5 days, (2) 0.5 mg/kg per day for 7 days, or (3) 0.25 mg/kg per day for 14 days (Random Allocation Software v1.0, Isfahan University of Medical Sciences,

Isfahan, Iran, 2004). After Peruvian malaria treatment guidelines, the maximum doses were 150 and 210 mg for the 5-day arm and the 7- and 14-day arms, respectively. In addition, all patients were scheduled to receive 25 mg/kg CQ over 3 days following Peruvian Ministry of Health (MoH) standards. PQ was administered on day 0 starting concurrently with the administration of CQ. Both CQ and PQ were dosed as the base form. The treatment allocation for each subject was placed in a sealed envelope, kept in an orderly manner, and opened only at the time of enrolling a new patient to prevent selection bias by study physicians.

CQ tablets (150 mg base) were divided into individual doses over 3 days: 10 mg/kg on the first and second days and 5 mg/kg on the third day. The tablets of PQ (15, 7.5, and 5 mg [each of the base form]) were divided into fractions down to a quarter as needed to provide the most accurate dose to each individual patient. All doses of CQ and PQ were administered along with food under the direct supervision of the study team. All subjects were observed for 30 minutes after treatment. If the subject vomited during this period, then he/she was retreated with the same dose of medication and observed for 30 additional minutes. Those patients who vomited a second time were dropped from the study. Severe and moderate adverse effects requiring hospitalization, including jaundice, hemolysis, or difficulty breathing, were recorded. Vomiting after treatment was also noted, and five symptoms were monitored during treatment days and all additional visits (fever, chills, headache, muscular pain, and dark urine). No other mild reactions were recorded. Both drugs were provided by the Peruvian MoH and procured through its standard provider (Laboratory CIPA S.A.). Concomitant treatment with paracetamol was used in case of fever. Subjects who admitted to taking any other antimalarial medication outside of the study were withdrawn from the study.

Thick blood smears were collected on days 2, 3, 7, 14, 21, and 28 and then every 15 days until day 210 counted from the day of enrollment (i.e., the first day of treatment). After treatment was completed (day 5, 7, or 14 depending on the study group), a member of the investigation team visited the residences of the volunteers two times per week until day 210 to take their

axillary temperature and inquire about the presence of any malarial symptoms, including fever or headache. Thick smears were collected if temperature was higher than 37.5°C or the subject had malaria-compatible symptoms. Also, subjects were encouraged to visit the clinic at any time if they felt ill, and additional thick smears were collected if subjects presented with fever or complained of any symptom of malaria. If any thick smear was positive for *P. vivax*, another sample of blood was taken in a tube with EDTA. For the purpose of the study, all recurrences were considered to be relapses, and follow-up was stopped. If the volunteer was febrile but the blood smear was negative, a new thick blood smear was taken every 24 hours until the fever remitted.

Smears between days 14 and 28 were taken with up to 2 days of margin before being considered lost to follow-up. After day 28, if subjects were not found at a scheduled bimonthly smear, they were visited daily, and if not found by the date of next scheduled thick smear, they were considered lost to follow-up.

Volunteers who developed recurrence of parasitemia at any time during the follow-up were treated with the treatment program recommended by the Peruvian MoH: CQ (25 mg/kg over 3 days) and PQ (0.5 mg/kg daily for 7 days). If they presented with *P. falciparum* malaria, mefloquine (12.5 mg/kg per day for 2 days) along with artesunate (4 mg/kg per day for 3 days) were administered according to the treatment program of the Peruvian MoH. The study team assured that the treatment was completed and followed up patients until their thick blood smears were negative. If the recurrence occurred between 17 and 35 days after initiating the standard treatment with CQ, a blood sample was collected on filter paper on the day of recurrence to evaluate the possibility of CQ failure, and the subject was excluded from the analysis. These results have been published elsewhere.¹⁸

Thick blood smears were stained with Giemsa 10% for 10 minutes and examined at 1,000× magnification to identify the species of parasite and determine the level of parasitemia. Parasite density was calculated by counting the number of asexual parasites per 200 white blood cells in the thick smear (assuming a mean white blood cell count of 6,000 per μ L). If more than 500 parasites were counted without counting 200 white blood cells, the count was stopped after reading the last oil immersion field containing the 500th parasite. In total, 300 oil immersion fields were read before a slide was considered negative. Gametocytes were not counted. Each blood smear was independently examined by two microscopists, and in the case of a discrepancy in results (positive/negative, species diagnosis, or more than twofold difference in parasite density), the blood smear was re-examined by a third independent microscopist. The final parasite density was reported as the average of the density readings from the two concordant microscopists.

The isolates of *P. vivax* collected on day 0 (before initiating therapy) and the day of parasite recurrence were genotyped to try to determine if parasitemia was caused by the same or a different strain in a secondary analysis. Five neutral microsatellite loci of substantial variability in the Peruvian Amazon Basin^{19,20} were determined to be good candidates to differentiate between whether the original infecting strain of *P. vivax* is genetically similar or different from the recurrent strain.²¹ In an area of sufficiently high genetic diversity, such as Iquitos,^{18,22} it can be expected that the possibility of a person being bitten on two separate occasions by two mosquitoes

carrying the same strain of *P. vivax* with exactly the same alleles at five or more loci would be very low. As such, if the initial and recurrent strains are genetically identical at all five microsatellite loci, the patient is very likely to be a true confirmed homologous relapse and a failure of PQ. However, in areas of emerging CQ resistance, such recurrences can also represent late recrudescences.

The primary outcome was the cumulative incidence of relapse (recurrence) between days 35 and 210, and in the secondary outcome, only confirmed homologous relapses were included. Relapse rates and other categorical variables were calculated as proportions and compared between study groups with χ^2 tests. Continuous variables were compared with the Kruskal–Wallis test or one-way analysis of variance. The risk of relapse over time was estimated through a Kaplan–Meier technique using all subjects who had at least some follow-up after day 35. The sample size was calculated to detect a difference of 10% in the relapse frequency (5% versus 15%) between the study groups, with 95% significance and 80% power. All calculations were conducted under an intention-to-treat approach with Stata v12 (Stata Corp., College Station, TX), and *P* values < 0.05 were considered significant.

RESULTS

During the period of the study, 29,927 patients were examined to rule out malaria at the three health centers. Of these patients, 2,670 patients had a thick blood smear positive for *P. vivax*; 1,873 of those patients (70.1%) were invited to enter the study, whereas the remaining 797 patients were diagnosed when researchers were absent. In total, 1,333 patients were excluded, primarily because they (1) lived too far from the community, which would make follow-up difficult, (2) had trips planned during the 6-month follow-up period, or (3) did not want to participate. Only two cases of G6PD deficiency were detected among 546 subjects tested (0.37%, 95% confidence interval [95% CI] = 0.04–1.31%). Ultimately, 540 patients were enrolled in the study: 294 patients in San Juan, 139 patients in Padre Cocha, and 105 patients in Santa Clara. Subjects were randomized into three groups of 180 patients each (Figure 2).

All of the enrolled cases had a fever at the time of enrollment or a history of fever within the previous 48 hours. The median age was 20 years (range = 1–77 years), and 53.3% were male. There was no statistical difference between the age, sex, weight, fever at time of enrollment, and parasite density between the three study groups (Table 1). The frequency of chills, headache, muscle pain, or dark urine at enrollment was also comparable (data not shown). The 10 heaviest participants (> 80 kg) received < 25% less of their weight-based PQ dose; one subject received a PQ dose 29% higher. Two patients vomited their medicine within 30 minutes (both in the 14-day arm) and were retreated. No one vomited a second time.

After enrollment, 45 patients (8.3%) were lost to follow-up (2 patients with incomplete treatment [both in the 14-day arm] and 9 patients before day 35). Eight additional patients (1.5%) were withdrawn from the study by the investigators. Two patients were excluded, because they took antimalarial medications outside the study without supervision; another two patients were excluded, because they presented with falciparum malaria during follow-up. Four patients were

FIGURE 2. Malaria cases and causes of exclusion.

withdrawn, because they presented a recurrence before 35 days. Three of the latter patients had subtherapeutic levels of CQ at the recurrence time, and the fourth recurrence had a 95-ng/mL CQ level (just below the 100 ng/mL required for confirmation of CQ failure). All four early recurrences were

considered probable CQ failures and excluded from the PQ relapse analyses, and they have been reported previously.¹⁸ There were no moderate or severe adverse reactions requiring hospitalization in any of the three study arms other than two pregnancies and a false-positive pregnancy test (all

TABLE 1
Characteristics of the patients enrolled in the three study groups

	5 days	7 days	14 days	P value
Study participants, <i>N</i>	180	180	180	
Median age, years	21.2	17.6	21.0	0.066
Age range, years	2–77	2–72	1–70	
Sex, % males	52.8	52.8	53.6	0.978
Axillary temperature $\geq 37.5^{\circ}\text{C}$, % (day 0)	36.6	31.7	31.7	0.172
Geometric mean parasite density, per μL (day 0)	7,151.5	6,685.8	7,166.0	0.652
Mean weight, kg	49.1	47.2	48.6	0.297
Weight range, kg	13.0–85.0	13.0–108.0	8.0–89.0	
Health facilities, %				0.992
Padre Cocha	26.1	25.6	25.6	
Santa Clara	20.0	18.3	20.0	
San Juan	53.9	56.1	54.4	
PQ daily dose received, mg/kg per day				
Median	0.51	0.50	0.25	< 0.001
Range	0.35–0.61	0.28–0.61	0.17–0.32	< 0.001
PQ total dose received, mg/kg				
Median	2.54	3.50	3.47	< 0.001
Range	1.76–3.06	1.94–4.29	2.36–4.53	< 0.001

TABLE 2

Cumulative incidence of relapse in patients with *P. vivax* infection treated with three different PQ regimens in the Peruvian Amazon Basin

	5 days	7 days	14 days
N	169	156	162
All relapses	48 (28.4%)	16 (10.3%)	22 (13.6%)
95% CI	21.7–35.8	6.0–16.1	8.7–19.8
Genotyping-adjusted relapses	26 (15.4%)	8 (5.1%)	12 (7.4%)
95% CI	10.3–21.7	2.2–9.9	3.9–12.6

All relapses: 5 versus 7 days, $P < 0.001$; 5 versus 14 days, $P = 0.001$; 7 versus 14 days, $P = 0.361$. Genotyping-adjusted relapses: 5 versus 7 days, $P = 0.003$; 5 versus 14 days, $P = 0.023$; 7 versus 14 days, $P = 0.403$.

observed months after treatment had finished). The 0.25 mg/kg per day dose arm (14 days) had higher rates of fever recorded on days 2 and 3 compared with the 0.50 mg/kg per day dose arms (5 and 7 days). The arms with higher daily PQ dose did not present significantly higher frequency of the five symptoms monitored during treatment.

Of 487 patients who completed follow-up until day 210, 86 patients showed recurrence of parasitemia after 35 days of initiation of therapy and are considered relapses for the purposes of our analyses (Table 2). The percent of subjects who relapsed by study group was 28.4% (48) in the 5-day treatment group (95% CI = 21.7–35.8%), 10.3% (16) in the 7-day treatment group (95% CI = 6.0–16.1%), and 13.6% (22) in the 14-day treatment group (95% CI = 8.7–19.8%). There was no statistically significant difference between the rate of relapse between those patients who received treatment of 7 and 14 days ($P = 0.361$), but there were statistically significant differences between those patients receiving 5 and 7 days of treatment, ($P < 0.001$) and those patients receiving 5 and 14 days of treatment ($P = 0.001$). We observed a borderline significant difference in the mean total PQ dose received by relapses and non-relapses in the low-dose 5-day arm (2.49 ± 0.26 versus 2.57 ± 0.25 mg/kg, $P = 0.063$), but no differences were found in the 7- ($P = 0.883$) and 14-day arms ($P = 0.926$).

The parasites in the initial and recurrent blood samples from all 86 cases of relapse (day 0 and day of recurrence) were

genotyped at five microsatellite loci. Of 86 cases, 46 cases presented with the same allele at each analyzed locus, and 40 cases were different in at least one locus (Table 2). Therefore, 53.5% of 86 relapses are considered as homologous relapses, whereas the rest (40) are either heterologous relapses or reinfections. The proportion of homologous relapses was similar in the three groups (range = 50–55%, $P = 0.953$). Again, there was no statistically significant difference in the adjusted rate of homologous relapse between those patients who received treatment of 7 and 14 days ($P = 0.403$), but the adjusted rate of homologous relapses in the 5-day regimen (15.4%) was significantly higher than in 7- (5.1%) and 14-day treatment regimens (7.4%).

Figures 3 and 4 show the timing of relapses in the three study groups based on the cumulative and instantaneous Kaplan–Meier hazard rates. The peak relapse period took place approximately between days 60 and 120, and the incidence of relapse dropped after 4 months in each of the three study arms ($P = 0.015$, $P = 0.065$, and $P < 0.001$).

DISCUSSION

In this study, we conclusively showed that a regimen of 150 mg total adult dose PQ (0.5 mg/kg for 5 days) was significantly less efficacious than two regimens of 210 mg total adult dose (either 0.5 mg/kg for 7 days or 0.25 mg/kg for 14 days). Additionally, our results confirm that the efficacy to prevent relapses of the 7-day regimen with double dosage (10.3%) is similar to the standard regimen of 14 days (13.6%). Finally, the absence of moderate and severe adverse effects in the study and the very low observed frequency of G6PD deficiency (0.4%) document that the 7-day 0.5 mg/kg regimen used by Peru and other countries in the region is a safe, effective, and well-tolerated treatment regimen.

Our findings are consistent with the literature describing that the adult PQ total dose of 210 mg base is superior to lower dosage regimens, despite the weaknesses of the available evidence.¹⁰ Similar results have been observed in the Americas. Almost all low (< 210 mg) PQ total dose studies consistently

FIGURE 3. Cumulative fraction of *P. vivax* infections that had not relapsed after treatment with three different regimens of PQ.

FIGURE 4. Instantaneous risk of relapse (smoothed hazard) in *P. vivax* infections treated with three different regimens of PQ.

report relapse rates of 20–30%^{13,14,23,24} and even 36–57%.^{25,26} Additionally, in our low-dose regimen, we observed that the actual dose received may be further associated with the risk of relapse. In contrast, full dose (210 mg base) clinical trials report relapse rates ranging from 0% to 10%^{13,23,27–30} and exceptionally, 15% and 18%.^{25,26} Relapse rates may vary between regions, which could affect the observed efficacy of PQ treatment, but using the WHO-recommended total dose of PQ clearly is more efficacious.

The relapse pattern observed after PQ treatment in our study seems to correspond to the early relapse observed in tropical vivax strains.³¹ Relapses started as early as 38 days after treatment initiation and peaked in the first 4 months. Few relapses were observed after day 150 in the most effective full-dose treatment arms, although we most likely did not capture all relapses. Follow-up stopped at 6 months, and a round of long-latency relapses could occur at 9–12 months but with greater chances of confounding because of reinfection. Therefore, the early relapse pattern observed suggests that studies of vivax radical cure in the Amazon Basin could be efficient and additionally, less affected by reinfection if focusing on short follow-up periods.

Our study was unable to conclusively discriminate relapses from reinfections among heterologous relapses. Relapses are common in the Amazon Basin (up to 40% in returning non-endemic travelers without reinfection risk).³² Relapses with different genotypes at one or more loci may be caused by (1) initial polyclonal infections with one or more clones undetected because of low density or (2) latent hypnozoites from previous infections.^{20,33} Both these factors can be common in our study area. Relapses are often caused by heterologous parasites, even in low transmission areas such as the Amazon Basin,^{15,31} and this result is consistent with the 11–70% probability of polyclonal infections reported in and around Iquitos.²² Additionally, reinfection and infection pressure are high in the Amazon basin: 57% recurrent heterologous infections primarily 2 months post-treatment were observed around Iquitos.³⁴ Branch and others³⁵ also estimated a similarly high incidence in Loreto, which was similar to findings

in previous PQ trials in South America. Therefore, reinfections probably account partially for heterologous recurrences in our study, most likely with similar frequency in all arms because of randomization (5.2–5.8% in full dose arms). We estimate that the relative efficacy of 7- over 5-day PQ probably is 61–78% depending on how much the force of reinfection accounts for recurrences. The absolute efficacy of the 7-day regimen compared with no relapse treatment is most likely higher, and its estimation most likely requires a detailed review of the regional literature.

Evidence suggests that a PQ dose of 0.5 mg/kg for 7 days is safe and well-tolerated in South America. No treatment-related hospitalizations were observed in our study. Also, the only few mild negative outcomes observed in the study were all in the 0.25 mg/kg per day dose (14-day) arm. Two subjects vomited after drug intake, two other participants did not complete treatment, and fever was more frequent in this group after 2–3 days of treatment. Additionally, the few mild adverse effects recorded in detail also concur with this statement. Other clinical trials in the region have also documented the safe intake of PQ at this dosage. Finally, ~607,000 cases of vivax malaria have been treated with this dose in Peru since 2000³⁶ with no reported events of toxicity or hemolysis and low mortality. Even considering the expected underreporting, this figure suggests a reasonably safe drug. Although we did not record sufficient data on mild adverse effects, the information available from our study and previous practice supports the possibility of testing higher daily doses of PQ for radical cure of *P. vivax* in South America.

Our study suffered from the traditional limitations of most clinical trials assessing the prevention of *P. vivax* relapses. Follow-up was limited to 6 months and interrupted after reaching the first observed vivax recurrence, reducing statistical power and preventing a thorough description of the impact of PQ on relapse patterns. Patients remained exposed to malaria, introducing reinfections that remained formally unaccounted. Finally, we lacked a study arm without PQ therapy that could have allowed assessment of the true reduction in the natural relapse rate of

P. vivax in the Amazon Basin. Several strategies were applied in the study design and implemented to deal with these issues. First, our sample size calculation targeted a small detectable difference, which was successfully observed, showing sufficient study power. Also, our efficacy estimates can be considered lower-bound figures, because the overall effect of the full dose of PQ is probably substantially larger if all additional relapses are taken into account. Second, although reinfections most likely have occurred, the study setting and observed distribution of homologous relapses suggest that reinfections do not affect our conclusions importantly. Additionally, regardless of whether the polymerase chain reaction-adjusted or unadjusted recurrences are used, the 5-day regimen is significantly less efficacious than either the 7- or 14-day regimens. Studying subjects with a single exposure would have been extremely challenging. Third, although it would have been ideal to have an arm with CQ alone to assess the vivax relapse rate in the Amazon basin, we did not include such an arm because of three practical and ethical concerns. First, our research question focused on the comparison of Peruvian therapy with the WHO recommendation of the time. Also, regional studies already describe high relapse rates when one-half dose or less PQ is provided.^{2,14,25,26,37} Finally, we found that an incomplete PQ regimen results in nearly 30% recurrence over 6 months, suggesting that studies with arms without radical cure drugs could induce preventable relapses.

The relapse rate in the 5-day regimen nearly tripled compared with the 7-day arm. More importantly, this very significant difference indicates that the total adult dose of 210 mg is at the very edge of reasonable efficacy. These results are free of confounding by the equalizing effect of randomization. The two additional daily doses seem to be critical, and partial compliance can severely undermine the clinical value of PQ at this dosage. Self-reported intake of all 7 days of the 0.5 mg/kg PQ dose ranged from 86% in Brazil, when all doses were handed at one time,³⁸ to 78% in Peru under directly observed therapy.³⁹ Despite the improved chances of compliance versus 14 days, 7-day treatment may still be far from ideal. In addition, 10% relapses in 6 months (or 5–25% as observed in Brazil and Colombia^{13,26,40}) are probably sufficient to maintain transmission in remote settings like the Amazon Basin and probably represent a major challenge to malaria elimination. PQ at higher total doses with shorter regimes may be an appealing alternative¹⁰ until other radical cure options, such as tafenoquine, are licensed and widely tested.⁴¹ The WHO recommends using higher doses, such as 0.5 mg/kg per day for 14 days, in southeast Asia and other regions where PQ tolerance has been shown.¹¹ The Centers for Disease Control and Prevention recently issued a similar recommendation for increased relapse prevention.⁴² Adult daily PQ doses of 60 mg were well-tolerated in Thai patients with normal G6PD,⁴³ and historical trials document the safe administration of high PQ doses with food.⁴⁴ Finally, the low prevalence of G6PD deficiency in the Peruvian Amazon Basin⁴⁵ may allow for the safe evaluation of improved PQ regimes that enhance both compliance and effectiveness, which have been advocated by Baird and Rieckmann.⁴⁶ Subsequent multicenter clinical trials in Amazonia could compare the standard 7-day 210-mg therapy with 270-, 330-, and even, 390-mg therapies delivered for 7 days or less. Therapies of up to 420 mg (with 30 mg two times per day for 7-day

regimens) have been tested safely in Thailand,⁴⁷ and a daily dose of 70 mg was safely administered together with food in Colombia.²⁵ Optimizing PQ therapy for the Amazonian countries could reach nearly 330,000 vivax cases per year and 74 million people at risk,¹ representing a major contribution for malaria control.

In conclusion, the regimen of 7 days of PQ with a dose of 0.5 mg/kg per day has a similar effect as the 14-day treatment of 0.25 mg/kg per day in its efficacy to prevent relapses during 210 days of follow-up, but treatment of 5 days with PQ at a dose of 0.5 mg/kg per day is less effective in preventing relapses. The current established malaria treatment guideline in Peru is effective to prevent relapses caused by *P. vivax* malaria. Future studies should evaluate whether shorter dosages with high total doses can be safe and effective in the Peruvian Amazon Basin, where G6PD deficiency rates seem to be very low.

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