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Short-course, high-dose primaquine regimens for the treatment of liver-stage vivax malaria in children

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ABSTRACT

Objectives: To assess the pharmacokinetics, safety, and tolerability of two high-dose, short-course primaquine (PQ) regimens compared with standard care in children with *Plasmodium vivax* infections.
Methods: We performed an open-label pediatric dose-escalation study in Madang, Papua New Guinea (Clinicaltrials.gov NCT02364583). Children aged 5-10 years with confirmed blood-stage vivax malaria and normal glucose-6-phosphate dehydrogenase activity were allocated to one of three PQ treatment regimens in a stepwise design (group A: 0.5 mg/kg once daily for 14 days, group B: 1 mg/kg once daily for 7 days, and group C: 1 mg/kg twice daily for 3.5-days). The study assessments were completed at each treatment time point and fortnightly for 2 months after PQ administration.
Results: Between August 2013 and May 2018, 707 children were screened and 73 met the eligibility criteria (15, 40, and 16 allocated to groups A, B, and C, respectively). All children completed the study procedures. The three regimens were safe and generally well tolerated. The pharmacokinetic analysis indicated that an additional weight adjustment of the conventionally recommended milligram per kilogram PQ doses is not necessary to ensure the therapeutic plasma concentrations in pediatric patients.
Conclusions: A novel, ultra-short 3.5-day PQ regimen has potential benefits for improving the treatment outcomes in children with vivax malaria that warrants further investigation in a large-scale clinical trial.

Introduction

Plasmodium vivax is the most widely distributed human malaria parasite with ~2 billion people at risk of infection and ~14.3 million clinical cases annually [1]. Although considered mild and nonlife-threatening, *P. vivax* is a major cause of morbidity and an under-recognized contributor to severe illness and death [2]. The infection is characterized by acute febrile illness, followed by recurrent episodes arising from relapsing dormant liver stages (hypnozoites) that are not cleared by most antimalarial drugs [3]. This presents unique challenges for case management and malaria control. An additional concern is that *P. vivax* variants from tropical regions relapse earlier and more frequently than strains from temperate zones. Because children carry the burden of repeated *P.vivax* infections in this geoepidemiologic setting [4], a safe, effective, and well-accepted radical cure would benefit both the patient and the broader community [5].

Primaquine (PQ), an 8-aminoquinoline, is one of the two drugs available for treating hypnozoites. However, its use has several challenges [6]. These include suboptimal adherence to recommended 14-day regimens [7], a lack of formulations and tablet strengths suitable for children, and few pharmacokinetic data in children to guide the evidence-based dosing [8,9]. Identifying

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the appropriate dosing regimens may also be influenced by intermediate or nonfunctioning liver cytochrome P450 isoenzyme CYP2D6 status, resulting in a decreased metabolism of PQ to bioactive metabolites and thus potentially lower the radical cure efficacy [10,11]. The high rates of glucose-6-phosphate dehydrogenase (G6PD) deficiency in vivax-endemic areas may prevent the routine use of oxidative drugs, including PQ. Severe hemolysis after PQ treatment in patients with G6PD deficiency is a major barrier to widespread deployment, necessitating preadministration G6PD testing. Furthermore, increasing evidence suggests that PQ metabolites formed through the cytochrome pathway correlate with methaemoglobin formation. Methaemoglobinaemia, the accumulation of clinically relevant concentrations of hemoglobin molecules unable to bind oxygen, can result from PQ administration and cause tissue hypoxia [12].

The cumulative PQ dose rather than the dosing schedule is considered the main determinant of efficacy [13]. Although the standard 14-day regimen of 0.25 mg/kg PQ daily (total 3.5 mg/kg) has an acceptable efficacy and attenuates the risk of hemolysis, it fails to prevent relapses in areas where the frequently relapsing strains are prevalent [14,15]. The World Health Organization (WHO) guidelines now include the use of a high-dose regimen of 0.5 mg/kg/day for 14 days (7 mg/kg total dose) in these areas based on the trials demonstrating improved efficacy in adults, especially when the dosing is supervised [14,16–18].

In most malaria-endemic settings, unsupervised 14-day PQ regimens are associated with reduced adherence and effectiveness [7] but individual dose supervision is impractical. Shorter courses of higher daily PQ doses do not appear to compromise efficacy and have the potential to improve adherence and thus effectiveness [19]. Two large, randomized controlled trials have shown that a 7-day regimen had a noninferior efficacy compared with the 14-day regimen [14,16]. In our previous studies in Melanesian children, we characterized the pharmacokinetic properties of a single-dose PQ and its primary metabolite carboxyprimaquine (CPQ). Based on the simulations using these data, we hypothesized that short (7 days of 1 mg/kg/day) and ultra-short (3.5 days of 1 mg/kg twice daily) PQ treatment regimens would be safe and well tolerated in this patient population [20].

The objectives of the current study were to assess the pharmacokinetics, safety, and tolerability of these abbreviated, high-dose PQ treatment regimens in Melanesian children infected with *P. vivax* malaria compared with the standard 14-day PQ treatment.

Methods

Study site, approvals, and patients

All clinical components were conducted within the Alexishafen Health Centre community catchment area, Madang Province, on the north coast of Papua New Guinea (PNG), where there is a hyperendemic malaria transmission. The recruitment of children with symptomatic (fever >37.5°C or history of fever in the previous 48 hours), uncomplicated slide positive vivax malaria, hemoglobin >80 g/l, and normal G6PD activity (BianaxNOW G6PD rapid diagnostic test [RDT], Abbott Australia]) was initially conducted from the health center outpatient clinic. Because the enrollment was slow due to the high ineligibility rates, the recruitment was expanded to children from the surrounding community. This latter group included children without symptoms severe enough to prompt presentation at the recruiting health center but who had a detectable acute blood-stage *P. vivax* infection by RDT (CareStart, AccessBio, Korea) that was confirmed by polymerase chain reaction (PCR) performed within 48 hours of sample collection. Only children who returned a normal G6PD screen and were *P. vivax*-positive (blood slide, RDT, and/or PCR) were eligible for enrollment. Children with features of severe malaria [17], a history of nonmalarial illness, PQ allergy, severe malnutrition, or moderate to severe anemia (hemoglobin <80 g/l) were excluded.

When the study was designed in 2009, there were no published PQ pharmacokinetic data in children, and only two known efficacy trials of short-course PQ regimens included children [21,22]. The current study was, therefore, designed to provide preliminary information that would inform a larger PNG pediatric PQ efficacy study. A sample size of 15 ($n = 12 \pm 20\%$) per dose group was considered sufficient for the pharmacokinetic evaluation and modeling based on the power calculations using the Monte-Carlo Mapped Power method automated through Perl-speaks-NONMEM (PsN; v7.2.0, ICON Development Solutions, Ellicott City, MD, USA). For the pilot safety evaluation, a total sample size of 40 was assumed as sufficient to characterize the common clinically concerning adverse events in each group. To allow the sequential interim analysis, the children were recruited into each of the first two treatment groups (group A: 0.5 mg/kg PQ for 14 days, and group B: 1 mg/kg PQ for 7 days) in 15 participant blocks, with an assessment of the safety and tolerability of these two regimens before the recruitment of children to the ultra-short–course regimen (group C: 1 mg/kg PQ twice daily for 3.5 days). The recruitment was ceased in 2017 because (i) the sample size was sufficient for a valid pharmacokinetic analysis, (ii) there were continued challenges identifying eligible participants, and (iii) the accumulated data were sufficient to inform a design of a large-scale clinical trial to further evaluate the short-course 3.5-day regimen [23].

The approvals for the current study were obtained from the PNG Institute of Medical Research Institutional Review Board (09.31) and the Medical Research Advisory Committee of the PNG Health Department (10.14), and the study was registered at Clinicaltrials.gov (NCT02364583).

Clinical procedures

Once recruited, each eligible participant was treated with a weight-based regimen of artemether-lumefantrine (AL; Coartem, Novartis) to clear the blood-stage parasites. Before each morning dose, a blood slide was prepared for microscopy with AL, then administered under direct supervision. The evening doses were supplied to the parent/guardian each day, with instructions for optimal administration.

Baseline assessments

The baseline assessment (before PQ administration) was conducted after the completion of AL treatment at least 3 days after enrollment (median, 3 days: range 3-8 days). This included a detailed history (including medical history, history of bed net usage, febrile illness, and malaria treatment within the previous fortnight), symptom questionnaire, and physical examination (weight, height, axillary temperature, midupper arm circumference, respiratory rate, blood pressure, and pulse rate).

A 2-ml venous blood sample was drawn for the preparation of malaria blood films and the measurement of hemoglobin (HemoCue, Ängelholm, Sweden), with the remainder of the sample immediately centrifuged. The packed red cells were stored frozen for PCR, and the plasma was used for the measurement of alanine transaminase, aspartate aminotransferase, total bilirubin, and creatinine (Dri-Chem NX500, Fujifilm Australia), with the remainder stored for drug assays. A urine sample was tested for the presence of protein, blood, and/or glucose. Baseline methemoglobin levels were determined by pulse oximetry (Masimo Rad-57 pulse oximeter with SpMet functionality; Masimo, Australia), and the corrected QT interval (QT_c) was measured from a 12-lead electrocardiogram (AT-101 ECG, Schiller Australia).

Treatment allocation

On day 0, the day of PQ treatment, the participants were allocated sequentially to one of three treatments in blocks of 15. Group A received 0.5 mg/kg PQ daily for 14 days, group B received 1 mg/kg PQ daily for 7 days, and group C participants received 1 mg/kg twice daily for 3.5 days (total PQ dose of 7 mg/kg in all groups). The participants were not required to fast. The doses were given as the whole or half of 7.5-mg tablets (PQ diphosphate; Shin Poon Pharmaceuticals, South Korea) under direct observation and given with water and food (biscuits) to minimize adverse gastrointestinal effects. If a child vomited within 1 hour of dosing, the same dose was readministered. Group C participants were admitted to the research ward for the duration of treatment for intensive safety monitoring.

As progression to the ultra-short–course regimen (group C) required a satisfactory interim analysis of the safety data from group B participants, the full sample size of 40 instead of 15 was recruited to group B. This deviation from the planned group size was implemented for two reasons. First, there was an unavoidable delay in the shipment of the clinical samples of group A and group B for pharmacokinetic evaluation. Second, the documentation detailing the exact sampling times required for the pharmacokinetic analysis in the first 23 participants was inadvertently lost before data entry. Although we were able to analyze the blood samples with a presumed collection time after PQ (median collection time of group A participants) as part of the safety analysis, this strategy was considered inappropriate for robust pharmacokinetic modeling at the time. All clinical and laboratory data collected for the 40 group B participants were available and used for pharmacokinetic modeling and to confirm the safety and tolerability before the recruitment progressed to group C.

Follow-up assessments

At each dosing time point, a side effect questionnaire, clinical examination, and signs and symptoms questionnaire were completed (Supplementary Table 1); methemoglobin levels were measured by pulse oximetry; and a finger-prick blood sample was collected for a blood film and measurement of hemoglobin. The 2-ml blood and urine samples were collected before dose 2 and 4 (all groups), dose 5 and 7 (group C), and the day after the last dose (day 15, 8, and 4 for group A, B, and C, respectively) for the hepatorenal function and dipstick urinalysis. Each participant was randomized to three pharmacokinetic sampling time points during the treatment regimen at which a 2-ml venous blood sample was collected immediately before the PQ dosing and 2-3 hours afterward (peak and trough drug concentrations). Each blood sample was immediately centrifuged (3000 × g), and the plasma was separated and stored at −80°C until analyzed.

After the completion of the PQ treatment, each participant attended fortnightly surveillance assessments for a total of 8 weeks for continued monitoring of the tolerability, safety, and efficacy outcomes (Supplementary material).

Pharmacokinetic analysis and modeling

The plasma concentrations of PQ and CPQ were determined simultaneously using a validated liquid chromatography–mass spectrometry method [20,24]. The intra- and interday precision for both PQ and CPQ were <10% across the concentration range of 5 to 1000 µg/l, with >85% recovery and a sensitivity of 1-2 µg/l. The $\log_e$ plasma concentration time data sets for PQ were analyzed by nonlinear mixed-effects modeling using NONMEM (v 7.2.0, ICON Development Solutions, Ellicott City, MD, USA), with an Intel Visual FORTRAN 10.0 compiler. Further details are provided in the Supplementary material.

Statistical analysis

Statistical analysis was performed using SigmaPlot for Windows Version 14.5 (Systat Software Inc, Germany). Data are summarized as means ± standard deviation or median and interquartile range (IQR), as appropriate. Two-sample comparisons for normally distributed variables were performed using the Student's *t*-test, for non-normally distributed variables, using the Mann-Whitney *U* test, and for proportions, using the Freeman-Halton extension of Fisher's exact test (two rows by three columns contingency table). Comparisons of continuous variables between the treatment groups were performed using the analysis of variance or Kruskal-Wallis test, as appropriate. Unless otherwise stated, all *P*-values are two-tailed, with $P < 0.05$ taken as significant.

Results

Patient characteristics

A total of 73 eligible children were recruited between August 2013 and May 2018 (Supplementary Figure 1). The parents of two children withdrew consent before PQ administration. The details of the remaining 71 children are summarized in Table 1. The three groups were well matched for most of the demographic, anthropometric, and clinical characteristics. Most patients (94%) had symptoms of uncomplicated malaria at recruitment, and 85% were slide positive for *P. vivax*. At baseline, age ($P = 0.009$), hemoglobin ($P = 0.029$), and methemoglobin ($P = 0.031$) concentrations differed significantly between the group A participants and group B and C participants. However, no participant had a baseline hemoglobin, methemoglobin, or biochemical parameters outside the normal Melanesian pediatric population reference ranges [25]. All participants were blood slide-negative for asexual forms at the time of PQ dosing. No child required retreatment due to vomiting. The mean daily PQ dose administered was 0.51 ± 0.08 mg/kg/dose (range: 0.42-0.70), 1.0 ± 0.11 mg/kg/dose (range: 0.77-1.3), and 0.98 ± 0.20 mg/kg/dose (range: 0.79-1.6) for group A, B, and C participants, respectively. Eight percent of the children received half-tablet fractions.

Safety

Methemoglobin concentrations increased by an average of 0.7%, 1%, and 2% from baseline for groups A, B, and C, respectively (Figure 1), but there was no significant between-group difference in median (IQR) methemoglobin on the day after the final PQ dose (1.7% SpMet [1.4-2.0%; range: 0.9-2.9%], 2.0% SpMet [1.4-2.8%; range: 0.9-4.5%], 2.0% SpMet [1.2-3.5%; range: 1.0-11.6%] group A, B, and C, respectively). Two participants in group C had methemoglobin levels >10% (10.1% and 11.6%) after the final dose but showed no signs of peripheral or central cyanosis, dizziness, or other symptoms of methemoglobinemia. All other group C participants reported methemoglobin levels <3.5%.

The hemoglobin concentrations fell during PQ therapy in all groups, with a median (IQR) decline to a nadir of 1 (-10–3) g/l, 0 (-6–7) g/l, and 5 (-11–1) g/l for group A, B, and C, respectively. Although the decline in hemoglobin was significant for group C participants ($P = 0.04$; nadir after PQ dose 4), there were no episodes of clinically significant hemolysis (>30% fall in hemoglobin) or related side effects. The median hemoglobin levels had returned to

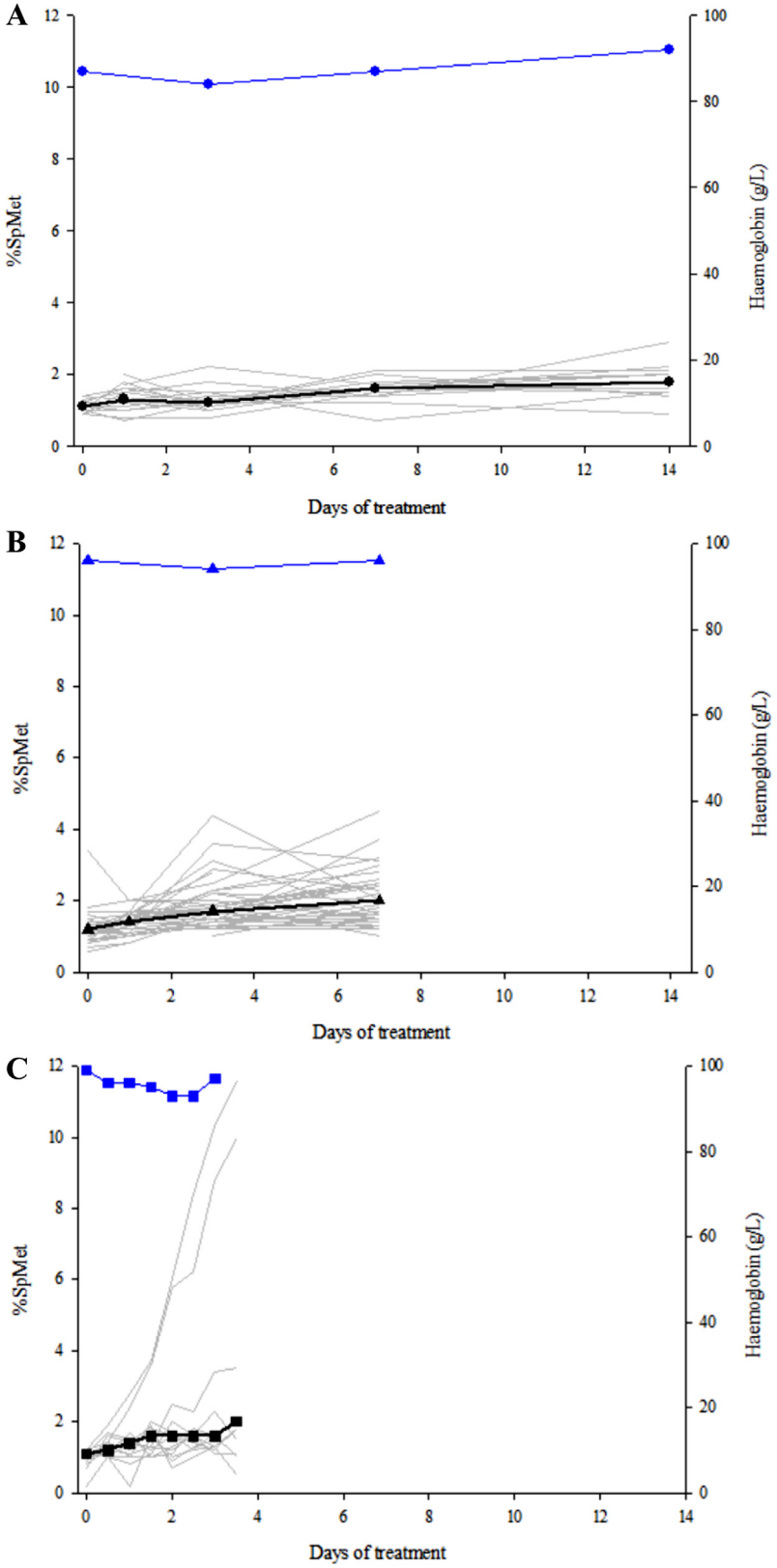

Figure 1. Hematologic safety outcomes. Median methemoglobin concentration (black; individual patient [gray]) and median hemoglobin concentration (blue) over treatment duration for 0.5 mg/kg PQ for 14 days (a), 1.0 mg/kg PQ for 7 days (b), and 1.0 mg/kg PQ twice daily for 3.5 days (c). PQ, primaquine.

Table 1
Demographic, clinical, and laboratory parameters. Data are presented as mean ± standard deviation, median (interquartile range), or percentage.

Parameter	Group A (n = 15)	Group B (n = 40)	Group C (n = 16)	*P*-value
Baseline (prior to dose 1)				
Age (years)	6.2 (5.0-7.7)	6.6 (5.7-7.8)	8.0 (6.9-9.0)	0.009
Symptomatic for malaria (%)	100	94	88	0.006
Vivax density by microscopy (parasites/µl)	1400 (142-5718)	1106 (127-3949)	296 (0-1574)	0.154
Sex (percent male)	60%	65%	56%	0.580
Body weight (kg)	17 (15-20)	18 (15-21)	18 (17-20)	0.470
Height (cm)	116 (108-119)	113 (109-121)	116 (112-118)	0.786
Axillary temperature (°C)	36.4 ± 0.3	36.3 ± 0.5	36.3 ± 0.6	0.644
Mid-upper-arm circumference (cm)	15.7 ± 1.5	15.7 ± 1.4	16.0 ± 1.2	0.545
Pulse rate (/min)	104 (80-108)	90 (84-99)	89 (82-97)	0.138
Respiratory rate (/min)	20 (18-20)	20 (18-22)	20 (20-24)	0.092
Hemoglobin (g/l)	87 (78-97)	96 (88-104)	99 (95-107)	0.029
Methemoglobin (%Sp)	1.1 (1.0-1.3)	1.2 (1.0-1.5)	1.1 (0.8-1.2)	0.031
QTc ($msec^{-1}$)	443 (404-448)	443 (424-454)	447 (433-456)	0.513
Serum creatinine (µmol/l)	26 (20-28)	26 (20-29)	22 (20-26)	0.691
Serum ALT (U/l)	20 ± 5.0	21 ± 5.1	22 ± 7.0	0.808
Serum AST (U/l)	29 (26-46)	29 (27-39)	33 (27-40)	0.849
Serum total bilirubin (µmol/l)	3.0 (3.0-3.5)	3.0 (3.0-3.0)	3.0 (3.0-3.0)	0.357
Before dose 2				
Pulse rate (/min)	100 (81-102)	87 (80-94)	84 (80-88)	0.061
Respiratory rate (/min)	20 (20-20)	20 (20-20)	20 (20-24)	0.095
Axillary temperature (°C)	36.4 ± 0.5	36.3 ± 1.9	36.2 ± 0.7	0.195
Methemoglobin (%Sp)	1.3 (1.0-1.6)	1.4 (1.1-1.6)	1.2 (1.0-1.5)	0.969
Serum creatinine (µmol/l)	23 (21-26)	26 (21-34)	22 (20-31)	0.595
Serum ALT (U/l)	21 (17-24)	21.5 (16-27)	23 (15-24)	0.959
Serum AST (U/l)	32 (26-39)	31.5 (27-38)	29 (25-33)	0.405
Serum total bilirubin (µmol/l)	3 (3-3)	3 (3-3)	3 (3-3)	0.645
Before dose 4				
Pulse rate (/min)	92 (88-100)	84 (80-92)	80 (80-86)	<0.001
Respiratory rate (/min)	20 (20-20)	20 (20-20)	20 (20-25)	0.022
Axillary temperature (°C)	36.4 ± 0.5	36.2 ± 0.7	36.1 ± 0.6	0.256
Hemoglobin (g/l)	84 (79-99)	94 (86-103)	93 (88-102)	0.121
Methemoglobin (%Sp)	1.2 (1.1-1.5)	1.7 (1.4-2.3)	1.6 (1.2-1.9)	0.006
QTc ($msec^{-1}$)	-	421 (400-441)	426 (411-433)	0.833
Serum creatinine (µmol/l)	24 (20-28)	25 (19-32)	21.5 (20-27)	0.667
Serum ALT (U/l)	21 (19-24)	20 (15-27)	20 (17-24)	0.801
Serum AST (U/l)	31 (26-37)	31 (29-38)	29 (25-33)	0.342
Serum total bilirubin (µmol/l)	3 (3-3)	3 (3-4)	3 (3-4)	0.053
Before dose 8				
(Day after PQ - B, C)				
Pulse rate (/min)	100 (90-101)	80 (80-88)	80 (75-88)	<0.001
Respiratory rate (/min)	20 (20-20)	20 (20-20)	20 (20-24)	0.030
Axillary temperature (°C)	36.2 ± 0.5	36.4 ± 0.5	36.3 ± 0.4	0.405
Hemoglobin (g/l)	87 (81-92)	96 (89-104)	100 (95-110)	0.006
Methemoglobin (%Sp)	1.6 (1.4-1.8)	2.0 (1.4-2.8)	2.0 (1.2-3.5)	0.144
Serum creatinine (µmol/l)		29 (23-34)	21 (19-22)	0.077
Serum ALT (U/l)		18 (15-22)	20 (17-26)	0.344
Serum AST (U/l)		31 (26-35)	32 (27-34)	0.582
Serum total bilirubin (µmol/l)		3 (3-6)	3 (3-4)	0.922
Day 15				
(Day after PQ - group A)				
Pulse rate (/min)	98 (88-100)	-	-	
Respiratory rate (/min)	20 (20-20)	-	-	
Axillary temperature (°C)	36.5 ± 0.4	-	-	
Hemoglobin (g/l)	92 (89-93)	-	-	
Methemoglobin (%Sp)	1.7 (1.4-2.0)	-	-	

ALT, alanine transaminase; AST, aspartate aminotransferase; PQ, primaquine; QT_c, corrected QT interval.

baseline levels by day 7 in all treatment groups (Figure 1 and Supplementary Table 3), with group A participants also showing significant hemoglobin recovery over the duration of the treatment ($P = 0.003$). Given the novelty of the ultra-short–course regimen, intensive safety monitoring was conducted before PQ doses 1, 3, 5, and 7 (Supplementary Table 3) but there were no significant changes relative to baseline in any of the measured parameter. There was no statistically significant difference in the comparison of most clinical safety parameters across the treatment groups between the time of enrollment and treatment completion (Table 1). No severe adverse events were reported.

Tolerability

All treatment regimens were relatively well tolerated, with 82.5% of the sample reporting no drug-related adverse effects (Table 2). The administration of 1 mg/kg PQ doses were associated with significantly increased reports of vomiting, abdominal pain, diarrhea, and anorexia compared with those receiving 0.5 mg/kg PQ doses. Those participants who received 1 mg/kg daily (group B) were more likely to report nausea ($P = 0.025$) and abdominal pain ($P = 0.008$) than those given 1 mg/kg twice daily (group C). All reported adverse events were mild (not disruptive of daily activi-

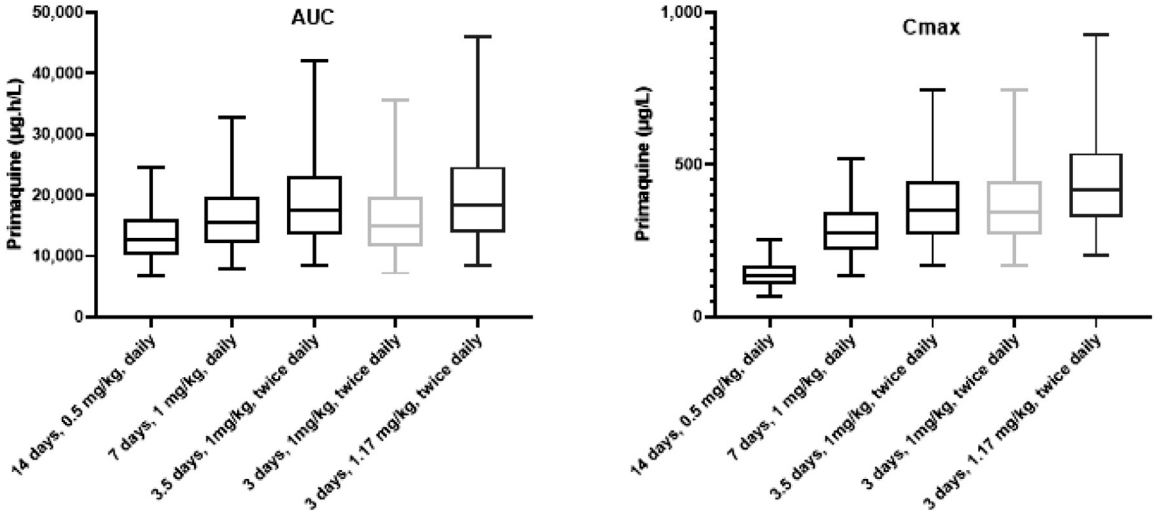

Figure 2. Box and whisker plots of AUC (a) and C_{max} (b) from simulations of various dose regimens based on the final model for 1000 patients for each kilogram of weight from 12-25 kg. Regimens not trialed in the current study are depicted in gray. AUC, area under curve.

Table 2
Number (percentages) of participants reporting side effects at least once during allocated primaquine treatment regimen.

	Group A (n = 15)	Group B (n = 40)	Group C (n = 16)	*P*-value
Nausea	0 (0)	2 (5)	0 (0)	0.012
Vomiting	0 (0)	4 (10)	1 (6.3)	0.007
Abdominal pain	1 (6.7)	7 (17.5)	1 (6.3)	<0.001
Diarrhea	0 (0)	3 (7.5)	1 (6.3)	0.001
Dizziness	0 (0)	0 (0)	0 (0)	1.000
Rash	0 (0)	1 (2.5)	0 (0)	0.110
Anorexia	0 (0)	3 (7.5)	1 (6.3)	0.001
Insomnia	0 (0)	0 (0)	0 (0)	1.000
Jaundice	0 (0)	0 (0)	0 (0)	1.000

ties) and did not extend beyond 24-hours or after two consecutive doses. No participant prematurely ceased treatment nor withdrew from the study due to the adverse effects.

Pharmacokinetic modeling and simulations

The population medians for V_{MAX} and K_M were 14,100 (µg/l)/h and 515 µg/l, respectively, equivalent to a clearance of 27.3 l/h per 70 kg at lower concentrations. A 1.7% reduction in relative bioavailability per day was estimated, corresponding to a 24% decrease over 14 days. The primary and secondary pharmacokinetic parameters are summarized in Table 3. The half-life ($t_{½}$) at low concentrations, given the Michaelis-Menten kinetics, were calculated from V_{MAX} and K_M, whereas the area under curve (AUC) up to 72 hours after the last dose was calculated using an additional compartment within NONMEM. The final parameter estimates and bootstrap results, including the main results from the multiple imputation, are summarized in Supplementary Table 6. Further details of the results from the model development and multiple imputation are presented in the Supplementary material.

The results from the simulation study are presented in Figure 2 and Supplementary Table 7. As expected, short-course regimens resulted in higher C_{max} values than the standard 14-day regimen. The AUC increased as treatment duration decreased, likely due to the Michaelis-Menten kinetics. The highest median estimated C_{max} (417 µg/l [95% simulation interval: 200-928]) and AUC (18,455 µg × h/l [8486-45,982]) was obtained when a total dose of 7 mg/kg was given as a 3-day regimen, with a 1.17-mg/kg twice-daily dose administration. A 3.5-day regimen of 1 mg/kg twice daily gave similarly high values. The simulation of a 3-day 1 mg/kg twice-daily regimen resulted in a similar drug exposure to a 7-day regimen (14,941 [7192-35,554] µg.h/l vs 15,470 [7832-32,657] µg.h/l, respectively) despite a lower total dose. Across all the simulated dosing regimens, weight was not associated with a significant variation in AUC (Supplementary Figure 4).

Discussion

This study provides evidence that a 3.5-day abbreviated high-dose PQ regimen is likely safe and well tolerated for the prevention of vivax malaria relapse in PNG children without G6PD deficiency. Supervised PQ doses given with food were not associated with gastroenterological side effects that would threaten adherence. Furthermore, potentially serious adverse effects of PQ (hemolysis and clinically significant methemoglobinaemia) were not observed. The simulations using pharmacokinetic data suggested that the duration of twice-daily 1 mg/kg doses could be reduced to only 3 days, without attenuating the drug exposure and thus efficacy (median simulated AUC of 15,470 µg.h/l, 17,514 µg.h/l, and 14,941 µg.h/l for 7-day 1 mg/kg daily, 3.5-day 1 mg/kg twice-daily, and 3-day 1 mg/kg twice-daily PQ regimens; Supplementary Table 7).

PQ and tafenoquine are the only drugs available for radical cure. Although the recent approval of a single-dose tafenoquine has significant implications for meeting the malaria elimination goals, the recent changes to the tafenoquine (Krintafel) label by the US Food and Drug Administration limit its approved use to coadministration with chloroquine [26]. This is a significant barrier for those vivax-endemic countries, including PNG, where artemisinin combination therapy (ACT) is recommended for blood-stage vivax due to chloroquine-resistant strains. Until tafenoquine is proven safe and efficacious for coadministration with ACT, PQ will continue to play a significant role in radical cure [17]. Optimized short-course PQ regimens have the potential to improve patient acceptability and adherence, with implications for its durability in malaria control programs.

The advantages of shortened PQ regimens are well recognized. Several studies evaluating their safety and efficacy in G6PD-normal individuals have found an improved treatment adherence and adequate drug exposure with a 7-day regimen (0.5 mg/kg PQ daily), providing an effective radical cure [8]. Our data demonstrate there is at least equivalent drug exposure in all simulated short-course regimens compared with the high-dose 14-day regimen (0.5 mg/kg

Table 3
Primary and secondary pharmacokinetic parameters for the individuals in the studies. Summarized as median [range] for each group.

Parameter	Historical 0.5 mg/kg single dose	Historical 1 mg/kg single dose	Group A	Group B	Group C
V_{MAX} (µg/l/h)	5995 [2994-11041]	5033 [2943-8879]	4447 [2843-8108]	4967 [2563-8307]	5209 [2979-8638]
K_M (µg/l)[a]	515	515	515	515	515
V/F (l)	60 -107]	59 [31-115]	53 [33-87]	52 [31-87]	53 [32-113]
ka (/h)	2.49 [0.51-4.91]	2.85 [0.48-4.92]	2.93 [2.16-4.2]	2.88 [1.26-21.92]	2.91 [0.64-6.39]
$t_{1/2}$ (h)[b]	3.57 [2.89-4.72]	3.65 [2.73-4.64]	3.72 [3.27-5.98]	3.72 [3.04-5.5]	4.02 [3.06-5.09]
AUC (µg.l/h)	877 [368-1547]	1889 [1220-2919]	12,685 [6390-20,416]	16,415 [10,138-43,106]	16,283 [10,476-49,944]

AUC, area under curve.
[a] No between subject variability in model, therefore same for all individuals.
[b] Calculated half-life at lower concentrations (i.e., << K_M).
[c] AUC from integration of differential equations within NONMEM to 72 hours after final dose.

PQ). In addition to the 3.5-day regimen, the modeling of a 3-day (1 mg/kg twice daily) PQ regimen also had a similar predicted drug exposure to the 7-day (1 mg/kg daily) regimen in children. The WHO recommends a 3-day course of ACT for blood-stage infection [17]. A PQ regimen of the same duration coadministered with ACT could represent an ideal treatment which eliminates blood-stage parasites, gametocytes, and hypnozoites. Where supervised treatment is not possible, a 3-day ACT-PQ regimen would likely improve adherence compared with the current recommendation of 3 days of ACT, followed by 14 days of PQ. However, high-dose PQ regimens (>1 mg/kg per dose) are often less well tolerated, with higher rates of gastrointestinal intolerance and methemoglobinemia [14,27]. Consumption of food at the time of PQ dosing is important in attenuating the gastrointestinal side effects and increasing the PQ exposure [28], but acute malaria can be associated with anorexia, nausea, and vomiting. Further studies of coadministered ACT-PQ are warranted in patients presenting with malaria in a vivax-endemic area, perhaps including the use of pretreatment antiemetics and antipyretics.

Abdominal pain is the most consistently reported side effect of PQ therapy. Although often mild and self-limiting, gastrointestinal symptoms can directly affect adherence. Significantly increased rates of gastrointestinal symptoms have been reported for 7-day (1 mg/kg) regimens [14,16]; although, they did not affect treatment adherence, with a noncompletion rate of 0.53% [14]. The current participants who received 1-mg/kg regimens similarly reported increased rates of gastrointestinal symptoms compared with the 0.5-mg/kg regimen. However, 82% of our sample reported no drug-related adverse effects, and no participants withdrew from the study due to tolerability concerns. Furthermore, increasing the frequency of dosing (twice daily) appeared to decrease the potential for side effects, with only participants receiving 1 mg/kg for 7 days self-reporting significantly higher rates of abdominal pain and nausea than those receiving 1 mg/kg twice daily for 3.5 days. We hypothesize that the admission to the health center for the period of PQ dosing and the close supervision of PQ dosing at the same time as food consumption may have attenuated the risk of these side effects in the 3.5-day PQ group.

The lack of hematologic and hepatorenal toxicity associated with the 3.5-day regimen is reassuring and consistent with findings from studies involving a 7-day regimen [29]. An initial modest decline in hemoglobin concentration is a well-recognized effect of PQ and was seen in each of our treatment groups. Similarly, there was a mean hemoglobin decline of 5 g/l at day 8 post-treatment in PNG children receiving 14-day 0.5-mg/kg daily PQ [30]. Reassuringly, those children who were symptomatic at time of recruitment showed hematologic recovery by day 15, as is expected after a successful treatment with ACT, with or without PQ [31].

A challenge for the design of short-course PQ regimens is the availability of pharmacokinetic data, which remain limited in children. Our initial PQ pharmacokinetic studies in healthy Melanesian children aged 5-12 years suggested little difference in the PQ exposure using conventional milligram/kilogram doses to that in adults [20]. However, this contrasts with studies in younger African [9], Brazilian [8], and Thai [32] children with malaria, who all showed a lower drug exposure. The therapeutic implication of these findings would be the necessity for children to have an additional weight adjustment of doses of PQ to ensure adequate plasma concentrations [8,9,20,32]. Our current and previous [20] data provide no justification for this strategy in children weighing between 12-25 kg and imply that the current 7-mg/kg dose regimens should be used in PNG, regardless of age, in accordance with the current national treatment guidelines.

The current study has limitations. Due to the unknown safety of the high-dose regimens at the time of recruitment, we used a highly conservative eligibility criteria, including hemoglobin concentrations >80 g/l. Because most PNG children acquire immunity to clinical infections with *P. vivax* during the first 5 years of life, the presence of low-density chronic infections without clinical symptoms is not uncommon [33,34]. Anemia is a direct consequence. This led to a significant proportion of screened children being ineligible (25% with confirmed vivax), resulting in the need for a modification of the recruitment strategy and a longer recruitment period, with implications for participant characteristics. Children in group A were generally younger, had higher respiration rates, and had significantly lower hemoglobin concentrations at recruitment than children in groups B and C. The sequential design and inadvertent delays in preventing the timely interim analysis of the safety data meant that group C children were recruited up to 2 years after those in group A. Although the sequential study designs can be validly used for phase 1 drug evaluation, a randomized trial design would have likely attenuated these issues. We had sufficient statistical power to provide insights into the pharmacokinetic parameters and simulation modeling, but robust assessment of safety, tolerability, and especially efficacy will require larger-scale studies.

The implementation of the current WHO-recommended 14-day PQ regimens have been hampered by poor acceptability and adherence and thus decreased efficacy, especially when taken without close supervision. An abbreviated treatment regimen is therefore likely to have significant benefits, if proven to be safe, well tolerated, and at least equally effective. Our novel, ultra-short 3.5-day

(1 mg/kg twice daily) PQ regimen demonstrated great promise in PNG children with confirmed G6PD activity (>30%). Further assessment of the safety, tolerability, and 84-day relapse efficacy of the 3.5-day regimen has recently been completed in PNG children [23]. Should the safety and efficacy of the regimen be demonstrated in other endemic settings when deployed with reliable point-of-care G6PD testing, this novel, short-course PQ regimen will represent a major advancement in malaria containment and eradication in vivax-endemic areas of the tropics.

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Ethical approval

The authors confirm that this study has been conducted with the ethical approval of all relevant bodies and is acknowledged in the manuscript.

Declarations of Competing Interest

The authors have no competing interests to declare.

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Author contributions

IB, IM, and TMED conceived and designed the study and were responsible for acquiring the funding. BRM managed the project administration and, in conjunction with ML, LJR, and TMED, provided supervision. MPS, SS, KTB, and BK developed and validated the analytical methods, while BRM, RT, JB, GY, BK, ML, LJR, and MPS were involved in the collection of research data. BRM and LM curated the data and, in conjunction with SS, conducted formal data analysis. BRM and SS drafted the manuscript, which was edited by LM, LJR, KTB, IM, and TMED. All authors reviewed the manuscript before its submission.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.05.063.

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