



Single dose primaquine for clearance of *Plasmodium falciparum* gametocytes in children with uncomplicated malaria in Uganda: a randomised, controlled, double-blind, dose-ranging trial



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Summary

Background Primaquine is the only available drug that clears mature *Plasmodium falciparum* gametocytes in infected human hosts, thereby preventing transmission of malaria to mosquitoes. However, concerns about dose-dependent haemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiencies have limited its use. We assessed the dose-response association of single-dose primaquine for gametocyte clearance and for safety in *P falciparum* malaria.

Methods We undertook this randomised, double-blind, placebo-controlled trial with four parallel groups in Jinja district, eastern Uganda. We randomly allocated Ugandan children aged 1–10 years with uncomplicated falciparum malaria and normal G6PD enzyme function to receive artemether–lumefantrine, combined with either placebo or with 0.1 mg/kg, 0.4 mg/kg, or 0.75 mg/kg (WHO reference dose) primaquine base. Randomisation was done with computer-generated four-digit treatment assignment codes allocated to random dose groups in block sizes of 16. Study staff who provided care or assessed outcomes and the participants remained masked to the intervention group after assignment. The primary efficacy endpoint was the non-inferiority of the mean duration of gametocyte carriage in the test doses compared with the reference group of 0.75 mg primaquine per kg, with a non-inferiority margin of 2.5 days. The primary safety endpoint was the superiority of the arithmetic mean maximum decrease in haemoglobin concentration from enrolment to day 28 of follow-up in the primaquine treatment groups compared with placebo, with use of significance testing of pairwise comparisons with a cutoff of $p=0.05$. The trial is registered with ClinicalTrials.gov, number NCT01365598.

Findings We randomly allocated 468 participants to receive artemether–lumefantrine combined with placebo (119 children) or with 0.1 mg/kg (116), 0.4 mg/kg (116), or 0.75 mg/kg (117) primaquine base. The mean duration of gametocyte carriage was 6.6 days (95% CI 5.3–7.8) in the 0.75 mg/kg reference group, 6.3 days (5.1–7.5) in the 0.4 mg/kg primaquine group ($p=0.74$), 8.0 days (6.6–9.4) in the 0.1 mg/kg primaquine group ($p=0.14$), and 12.4 days (9.9–15.0) in the placebo group ($p<0.0001$). No children showed evidence of treatment-related haemolysis, and the mean maximum decrease in haemoglobin concentration was not associated with the dose of primaquine received—it did not differ significantly compared with placebo (10.7 g/L, SD 11.1) in the 0.1 mg/kg (11.4 g/L, 9.4; $p=0.61$), 0.4 mg/kg (11.3 g/L, 10.0; $p=0.67$), or 0.75 mg/kg (12.7 g/L, 8.2; $p=0.11$) primaquine groups.

Interpretation We conclude that 0.4 mg/kg primaquine has similar gametocytocidal efficacy to the reference 0.75 mg/kg primaquine dose, but a dose of 0.1 mg/kg was inconclusive for non-inferiority. Our findings call for the prioritisation of further trials into the efficacy and safety of doses of primaquine between 0.1 mg/kg and 0.4 mg/kg (including the dose of 0.25 mg/kg recently recommended by WHO), in view of the potential for widespread use of the drug to block malaria transmission.

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Introduction

Effective drug therapy is a key component of malaria control and elimination strategies to reduce both morbidity from the disease and onward transmission to mosquitoes.¹ Artemisinin combination therapy (ACT), the first-line treatment in sub-Saharan Africa, achieves excellent cure rates for *Plasmodium falciparum* through rapid clearance of the asexual stages of the parasite. As a consequence, ACT reduces the production of malaria

transmission stages—gametocytes—and thereby restricts transmission potential.² However, onward malaria transmission is not completely prevented because of the inadequate effect of artemisinins and their partner drugs against mature gametocytes. If mature gametocytes are present before treatment, they persist after ACT, often at concentrations below the threshold for detection by conventional microscopy,³ and can allow onward malaria transmission for up to 14 days after treatment.^{3–6}

Primaquine, an 8-aminoquinoline, is the only available drug with established activity against mature gametocytes. It clears circulating gametocytes that persist after ACT, thereby reducing the duration of gametocyte carriage,^{7–12} and renders most patients free of gametocytes by day 14 after initiation of ACT–primaquine treatment.^{7–9,12} Primaquine reduces the transmission of malaria to mosquitoes—an effect that might precede the clearance of gametocytes.^{13,14} The transmission-blocking properties of primaquine have been reviewed in detail.¹⁵ WHO has recommended one dose of primaquine in addition to ACTs for use in two scenarios: for malaria elimination programmes, and to stop the spread of emerging artemisinin resistance.¹⁶ Primaquine is recommended for use in first-line antimalarial treatment in many countries.¹⁷

Despite these recommendations, primaquine is often not used because of concerns about its haemolytic effect in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Primaquine-induced haemolysis can occur after one dose of the drug¹⁸ and is dose dependent.¹⁹ Because doses of primaquine lower than the WHO-recommended dose can be equally efficacious at clearance of *P. falciparum* gametocytes,¹⁵ dose optimisation for ACT–primaquine is needed.

No formal randomised controlled trials have been done to characterise the dose-response relation of primaquine for *P. falciparum* gametocyte clearance. We aimed to assess the efficacy of reduced doses of primaquine for non-inferiority to the WHO reference dose of 0.75 mg primaquine base per kg that has proven efficacy,^{7,20} and to assess for superiority of the safety of reduced doses compared with placebo, in people with normal G6PD enzyme function.

Methods

Study design and participants

The study was a randomised, double-blind, placebo-controlled trial with four parallel groups. The study protocol has been described in detail elsewhere.²¹ Briefly, we undertook the study at Walukuba Health Centre IV in Jinja district, eastern Uganda, between December, 2011, and March, 2013. In this region, malaria transmission is perennial with seasonal peaks in intensity. An entomological inoculation rate of seven infectious bites per person per year was estimated in 2001.²²

Eligible participants were children aged 1–10 years attending the health centre with fever or history of fever in the past 24 h, *P. falciparum* mono-infection with a parasite density lower than 500 000 per μL , and normal G6PD enzyme function based on a fluorescence spot test (R&D Diagnostics, Aghia Paraskevi, Greece). Exclusion criteria were evidence of severe illness or danger signs, haemoglobin concentration less than 80 g/L, known allergy to the study drugs, antimalarials taken within the past 2 days, primaquine taken within the past 4 weeks, and blood transfusion within the past 90 days. Written

informed consent was provided by parents or guardians and, in addition, assent was provided by children older than 8 years of age.

Ethics approval for the trial protocol and informed consent forms were provided by the Makerere University School of Medicine research ethics committee (protocol 2011-210), the Uganda National Council of Science and Technology (protocol HS1056), and the London School of Hygiene and Tropical Medicine research ethics committee (protocol 5987). The Ugandan National Drug Authority approved importation of the study drug. The trial data safety monitoring board and trial advisory committee were convened before the start of the trial and met at predetermined stages of the study. Consultations with local community stakeholders in Walukuba were held before, during, and after trial completion.

Randomisation and masking

We randomly assigned eligible participants to one of four dose groups. In each group, we gave participants artemether–lumefantrine twice daily on days 0–2 and, with the fifth dose of the drug, one dose of either placebo or primaquine (0.1 mg/kg, 0.4 mg/kg, or 0.75 mg/kg). A statistician at the London School of Hygiene and Tropical Medicine (ELW) computer-generated four-digit treatment assignment codes and allocated these to random dose groups in block sizes of 16. To achieve treatment concealment, we added masking syrup to all treatment groups, which disguised the colour and taste of the study drug. Because G6PD deficiency is an X chromosome-linked disorder, we stratified randomisation by sex. Sequential sealed envelopes containing a randomisation code were selected by the study pharmacist from either the male or female pile. The pharmacist was not involved in patient outcome assessment. All other study staff providing care or assessing outcomes, and the participants themselves, remained masked to the intervention group after assignment.

Procedures

We crushed 15 mg base primaquine phosphate tablets and dissolved them in 15 mL of drinking water to produce a stable 1 mg/mL solution. We drew up the assigned dose to the nearest 0.5 mL through a sterile syringe and immediately gave it to each participant in a plastic cup or spoon. We administered all treatments after the children had eaten a fatty snack (biscuits) and then directly observed the patients. If a child vomited within 30 min, treatment was re-administered. Those who vomited more than three times were excluded from the study and were treated for complicated malaria.

Enrolled participants were reviewed on days 0, 1, 2, 3, 7, 10, 14, 21, and 28, or on additional days if they presented at the clinic. We did systematic and prospective assessments for adverse events. We graded new or worsening symptoms, examination findings, or laboratory abnormalities according to a severity scale (adapted from

the WHO toxicity grading scale for determining the severity of adverse events and from the National Institutes of Health, Division of Microbiology and Infectious Diseases paediatric toxicity tables published in January, 2003)²³ and assessed causal associations with the study drug. We implemented a standardised protocol to detect episodes of haemolytic anaemia, which we have published elsewhere.²¹ On scheduled visits, we collected roughly 500 µL of venous blood for laboratory assessments. On all visits, we did asexual malaria parasite counts, in which we enumerated parasites per 200 white blood cells; we read 100 microscopy fields in the Giemsa-stained thick blood film before we judged a slide to be parasite negative. At enrolment, we read slides twice specifically for gametocytes, following the same procedure as that for asexual parasites. We measured haemoglobin concentration on days 0, 1, 2, 3, 7, 10, 14, 21, and 28 with self-calibrating HemoCue 201+ photometers (HemoCue; Angelholm, Sweden). We assessed gametocytaemia by quantitative real-time nucleic acid sequence-based analysis (QT-NASBA) with *Pf*s25 mRNA²⁴ on days 0, 2, 3, 7, 10, and 14. The timing of gametocytaemia measurements was based on findings from previous studies that suggested the gametocyte-clearing effect of primaquine is restricted to the first 2 weeks after treatment.^{7,25} We extracted nucleic acids from 50 µL blood samples in L6 buffer (Severn Biotech Limited, Kidderminster, UK) with Total Nucleic Acid Isolation Kits–High Performance (Roche Applied Science, Mannheim, Germany) and a MagNA Pure LC automated extractor (Roche Applied Science). The sensitivity of this assay is related to the volume of blood sampled and is in the range of 0.02–0.1 gametocytes per µL for the samples collected.²⁴

The primary endpoint for efficacy was the non-inferiority of the mean duration of gametocyte carriage in the test doses compared with the reference group of 0.75 mg primaquine base per kg. Secondary endpoints were the point prevalence of gametocytes on days 7, 10, and 14 after treatment, gametocyte circulation time, and the area under the curve (AUC) of gametocyte density over time after primaquine administration. For treatment outcomes in each group, definitions of adequate clinical and parasitological response, early treatment failure, and late treatment failure were according to WHO Methods for Surveillance of Antimalarial Drug Efficacy.²⁶ The primary safety endpoint was the superiority of the arithmetic mean maximum decrease in haemoglobin concentration from enrolment to day 28 of follow-up in the primaquine treatment groups compared with the placebo group. Secondary safety endpoints were the superiority assessment of the day of haemoglobin nadir, the maximum percentage decrease in haemoglobin, the percentage of participants with haemoglobin concentration lower than 50 g/L, requirement for blood transfusion, evidence of black urine, and the frequency of severe adverse events.

Statistical analysis

In our sample size calculation, we took into consideration the primary endpoints for both efficacy and safety. To guide the efficacy calculation, we used the QT-NASBA-measured duration of gametocyte carriage in a Tanzanian study, which was reduced from a mean of 28.6 to 6.3 days (SD 6) when primaquine (0.75 mg/kg) was added to ACT alone.²⁵ Efficacy analyses were done on an intention-to-treat basis. To assess non-inferiority of the test groups to the reference group with 80% power at the two-tailed 5% significance level, with allowance for 10% loss to follow-up and with use of a proposed clinically relevant non-inferiority margin of 2.5 days, the target sample size for efficacy was 120 participants per group. However, during the course of review by the trial data safety monitoring board, the target sample size was reduced to 460 participants (ie, 115 per group instead of 120) because of a lower than expected loss to follow-up. For the safety component of our analysis, the sample size calculation was based on the mean decrease in HemoCue-measured haemoglobin concentration on day 7 after treatment with primaquine of 6 g/L (SD 15) in a previous Tanzanian study.¹⁸ A sample size of 99 participants per group would provide 80% power to detect a difference in mean maximum decrease in haemoglobin between treatment groups of 6 g/L at a significance level of 5%.

Data were double entered and transferred into Stata (version 12.0) for analysis. We estimated duration of gametocyte carriage and gametocyte circulation time in children with gametocytaemia on day 2 (the day of primaquine dosing) with a straightforward deterministic compartmental mathematical model²⁵ that allows for the release of gametocytes from sequestration and incorporates baseline gametocyte densities into model estimates. The model allows the duration of gametocyte carriage to be estimated as a continuous outcome. As the spacing between sampling times increases, some uncertainty is expected, but this was judged to be acceptable for estimates during the first 14 days after initiation of treatment. We compared treatment groups for non-inferiority to the reference group with two-sided 95% CIs. Because the distribution of gametocyte densities was expected to be skewed, all density analyses involved log10-transformed data and we used geometric means as summary statistics. We assessed the AUC of gametocyte density per participant with the linear trapezoid method²⁷ and log10-transformed the data. We used ANOVA to compare log AUC with the reference treatment group. We compared gametocyte point prevalence estimates per treatment group with the reference group with use of the prevalence ratio with 95% CIs. We adjusted all efficacy analyses for gametocyte density at enrolment, and tested the potential effect of sex by adding this variable to multivariate models and by doing a stratified analysis.

The primary safety outcome, maximum decrease in haemoglobin (g/L) during follow-up compared with the measurement at enrolment, is expressed as an arithmetic

mean per treatment group and pairwise comparisons made between placebo and each of the primaquine groups, with unpaired *t* tests. We used a cutoff for significance tests of $p=0.05$ for the superiority analysis. We compared the occurrence of adverse events between groups; the significance level was adjusted for several comparisons by Bonferroni correction. This trial is registered with ClinicalTrials.gov, number NCT01365598.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study. All authors reviewed the report and agreed to submit for publication.

Results

We screened 1215 children with a history of fever and a positive blood smear at Walukuba Health Centre for eligibility to enrol in the study. The most frequent reason for exclusion was having taken antimalarial drugs in the

previous 48 h (figure 1). Between December, 2011, and December, 2012, we enrolled and randomly allocated 468 children, 461 of whom completed treatment and contributed data for the assessment of safety and efficacy (figure 1). 36 of these 461 children (8%) did not complete 28 day follow-up. The proportion lost to follow-up did not differ significantly between treatment groups, but was highest in the placebo group (figure 1). Baseline characteristics were similar in all treatment groups (table 1). 199 of 461 (43%) children were anaemic at baseline (haemoglobin concentration <110 g/L). Treatment failure, assessed clinically and microscopically, was rare (table 2) and did not differ significantly between groups ($p=0.68$).

Gametocyte prevalence at enrolment was 22.6% (104/461) by microscopy and 81.8% (365/446) by QT-NASBA (table 1), and did not differ between treatment groups ($p=0.91$ for microscopy and $p=0.42$ for QT-NASBA). Gametocyte density at enrolment was numerically higher in the 0.75 mg/kg reference group (table 1) but did not differ significantly from any of the other groups ($p\geq 0.31$). Gametocyte prevalence decreased

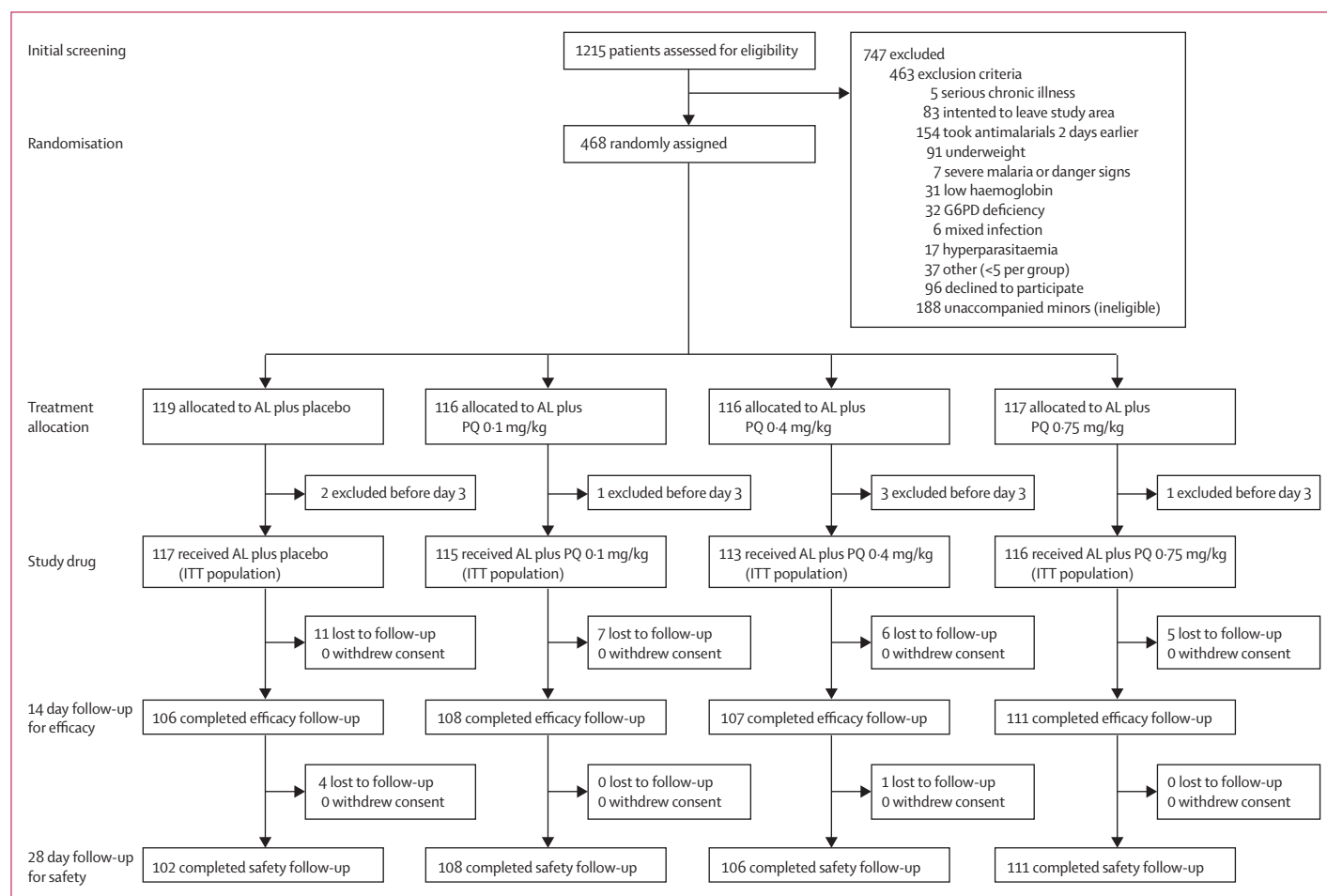


Figure 1: Trial profile

AL was given as six doses over 3 days (days 0, 1, and 2); PQ or placebo was given together with the fifth dose of AL on the morning of day 2. The two post-treatment exclusions in the 0.4 mg/kg treatment group (because of delayed confirmation of parasitaemia) were followed up for safety. G6PD=glucose-6-phosphate dehydrogenase. AL=artemether-lumefantrine. PQ=primaquine. ITT=intention to treat.

	Placebo (n=117)	Primaquine 0.1 mg/kg (n=115)	Primaquine 0.4 mg/kg (n=113)	Primaquine 0.75 mg/kg (n=116)
Boys	48.7% (57/117)	49.6% (57/115)	49.6% (56/113)	49.1% (57/116)
Age (years)	5.0 (3.0–7.5)	5.0 (3.3–7.0)	5.3 (3.2–7.0)	4.1 (3.0–7.0)
Bodyweight (kg)	16.0 (13.0–20.5)	16.0 (13.0–22.0)	17.0 (14.0–23.0)	15.0 (13.0–19.0)
Body temperature (°C)	38.0 (1.0)	38.3 (1.1)	38.0 (1.2)	38.2 (1.1)
Haemoglobin concentration (g/L)	113 (15)	109 (15)	112 (15)	112 (14)
Geometric mean sexual parasite density, parasites/mL (IQR)	17 661 (5260–65 130)	18 420 (4440–92 780)	16 457 (3260–81 240)	32 497 (10 880–151 180)
Gametocyte prevalence by microscopy	23.1% (27/117)	24.3% (28/115)	20.4% (23/113)	22.4% (26/116)
Gametocyte prevalence by QT-NASBA	79.8% (91/114)	86.7% (98/113)	78.7% (85/108)	82.0% (91/111)
Geometric mean gametocyte density (gametocytes/ μ L) by QT-NASBA (IQR)	15.2 (8.4–27.8)	14.5 (8.9–23.5)	19.4 (11.3–33.1)	24.6 (14.9–40.5)

Data are % (n/N), median (IQR), or mean (SD), unless otherwise indicated. QT-NASBA=quantitative real-time nucleic acid sequence-based analysis.

Table 1: Baseline characteristics

	Placebo	Primaquine 0.1 mg/kg	p value*	Primaquine 0.4 mg/kg	p value*	Primaquine 0.75 mg/kg	p value*
Number evaluated	117	115	..	113	..	116	..
Excluded from ITT analysis							
Withdrawal unrelated to study drug or malaria	0	0	..	2/113 (1.8%)	0.245	0	..
Lost to follow-up	15/117 (12.8%)	7/115 (6.1%)	0.080	7/113 (6.2%)	0.088	5/116 (4.3%)	0.033
ACPR on day 28	98/102 (96.1%)	101/108 (93.5%)	0.41	106/106 (100%)	0.12	106/111 (95.5%)	0.83
Treatment failures							
Early (day 3)	0	0	..	0	..	0	..
Late (day 28)	4/102 (3.9%)	7/108 (6.5%)	0.41	0	0.12	5/111 (4.5%)	0.83

Data are n/N (%), unless otherwise indicated. ITT=intention to treat. ACPR=adequate clinical and parasitological response. Definitions of ACPR, early treatment failure, and late treatment failure are according to WHO Methods for Surveillance of Antimalarial Drug Efficacy 2009.²⁶ *p values are for comparison with placebo, with χ^2 or Fisher's exact tests. Outcomes are unadjusted by PCR.

Table 2: Treatment outcomes for the different regimens on day 28 after start of treatment

	Placebo	p value*	Primaquine 0.1 mg/kg	p value*	Primaquine 0.4 mg/kg	p value*	Primaquine 0.75 mg/kg
Duration of gametocyte carriage (days) [†]	12.4 (9.9–15.0)	<0.0001	8.0 (6.6–9.4)	0.14	6.3 (5.1–7.5)	0.74	6.6 (5.3–7.8)
Circulation time per gametocyte (days)	1.97 (1.64–2.31)	<0.0001	1.47 (1.22–1.73)	0.0012	0.95 (0.77–1.13)	0.80	0.98 (0.78–1.18)
Gametocyte prevalence on day 7	40/115 (34.8%)	0.001	25/108 (23.1%)	0.044	11/104 (10.6%)	0.47	15/104 (14.4%)
Gametocyte prevalence on day 10	23/112 (20.5%)	0.008	18/107 (16.8%)	0.020	10/107 (9.3%)	0.46	8/108 (7.4%)
Gametocyte prevalence on day 14	16/105 (15.2%)	0.017	6/103 (5.8%)	0.72	3/103 (2.9%)	0.51	6/106 (5.7%)

Data are mean (95% CI) or n/N (%). Except for the duration of gametocyte carriage, all estimates were adjusted for gametocyte density at enrolment. *p values are for comparison with reference 0.75 mg/kg treatment group. [†]Calculated for all children who had gametocytes on the day of primaquine or placebo administration.

Table 3: Gametocyte carriage during follow-up for the different treatment regimens

after enrolment, although 170 of 345 (49.3%) participants who were gametocyte positive at enrolment remained so on day 2 before receiving primaquine or placebo. After day 2, the rate of gametocyte clearance was dependent on treatment group. The mean duration of gametocyte carriage was 6.6 days (95% CI 5.3–7.8) in the 0.75 mg/kg reference group, 6.3 days (5.1–7.5) in the 0.4 mg/kg group, 8.0 days (6.6–9.4) in the 0.1 mg/kg group, and 12.4 days (9.9–15.0) in the placebo group (table 3). The duration of gametocyte carriage for children who were

gametocyte positive at primaquine administration was the primary outcome and was tested for non-inferiority to the 0.75 mg/kg reference group. With the proposed non-inferiority margin of 2.5 days, the 0.4 mg/kg group showed non-inferiority to the reference 0.75 mg/kg group, but the 0.1 mg/kg group was inconclusive for non-inferiority and placebo was inferior (figure 2).

The mean circulation time of gametocytes indicated a longer circulation time of gametocytes in the 0.1 mg/kg group ($p=0.0012$) and the placebo group ($p<0.0001$) than

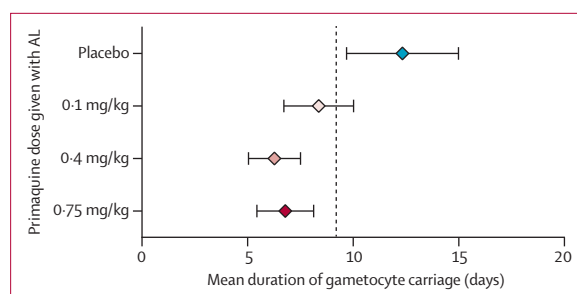


Figure 2: Mean duration of gametocyte carriage by treatment regimen

The duration of gametocyte carriage was estimated by fitting of a straightforward deterministic compartmental mathematical model to repeated *Pfs25* quantitative real-time nucleic acid sequence-based analysis gametocyte prevalence estimates. Symbols indicate the mean duration of gametocyte carriage, and error bars represent the upper and lower limit of the 95% CI. The dashed line indicates the set threshold for non-inferiority compared with the 0.75 mg/kg reference group (non-inferiority margin of 2.5 days). AL=artemether-lumefantrine.

in the reference 0.75 mg/kg group (table 3). Gametocyte circulation time did not differ significantly between the 0.4 mg/kg group and the reference 0.75 mg/kg group ($p=0.80$). Compared with the reference 0.75 mg/kg group, gametocyte prevalence was significantly higher in the 0.1 mg/kg group on days 7 and 10, and significantly higher in the placebo group on days 7, 10, and 14 (table 3). We recorded no difference in prevalence between the 0.4 mg/kg group and the reference group throughout follow-up (table 3, figure 3). The overall geometric mean gametocyte density was 17.9 gametocytes per μL (95% CI 13.8–23.3) at enrolment, 15.7 gametocytes per μL (11.0–22.2) on day 2 before primaquine treatment, 11.6 gametocytes per μL (7.2–18.8) on day 3, 5.3 gametocytes per μL (3.0–9.3) on day 7, 5.2 gametocytes per μL (2.6–10.5) on day 10, and 2.1 gametocytes per μL (0.7–5.7) on day 14. This decrease in the density of gametocytes in gametocyte-positive people during follow-up was statistically significant ($p<0.0001$) but densities in these patients did not differ significantly between treatment groups on discrete follow-up days (data not shown).

The AUC of gametocyte density over time, a measure that incorporates both prevalence and density of QT-NASBA estimates, was 3.8 (95% CI 1.7–8.2) gametocytes per μL per day in the placebo group, 3.8 (1.8–7.8) in the 0.1 mg/kg group, 2.1 (1.0–4.5) in the 0.4 mg/kg group, and 2.0 (0.9–4.3) in the 0.75 mg/kg group. After adjustment for gametocyte density at enrolment, the AUC compared with the reference group did not differ significantly for the 0.4 mg/kg group ($p=0.79$) or the placebo group ($p=0.16$), but was significantly higher in the 0.1 mg/kg group ($p=0.043$; data not shown). None of the efficacy estimates were affected by the sex of the participants (data not shown).

The mean maximum decrease in haemoglobin concentration did not differ significantly compared with placebo (10.7 g/L, SD 11.1) in the 0.1 mg/kg (11.4 g/L, 9.4; $p=0.61$), 0.4 mg/kg (11.3 g/L, 10.0; $p=0.67$), or

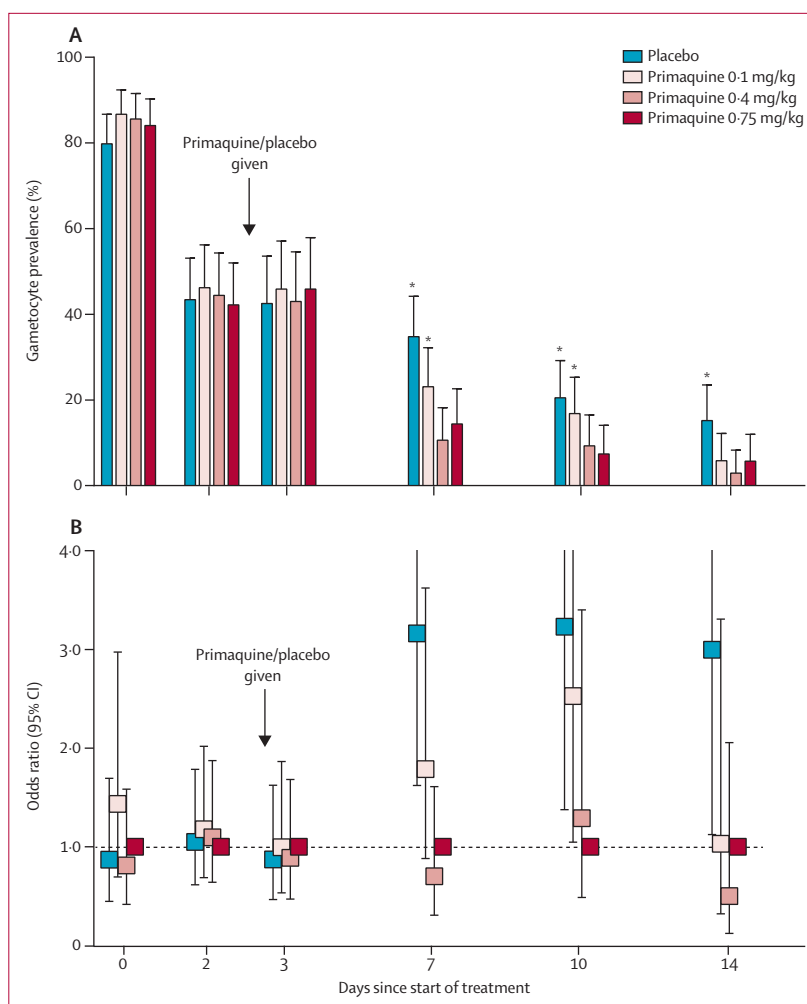


Figure 3: Gametocyte prevalence and prevalence ratio for each treatment regimen during 14 day follow-up

(A) Gametocyte prevalence during follow-up, as measured by *Pfs25* quantitative real-time nucleic acid sequence-based analysis. Error bars indicate the upper limit of the 95% CI. (B) Odds ratio of gametocyte prevalence on each of the days of follow-up compared with the reference 0.75 mg/kg group after adjustment for baseline gametocyte density. Error bars indicate the upper and lower limits of the 95% CI. *Indicates a statistically significant difference compared with the reference 0.75 mg/kg group.

0.75 mg/kg (12.7 g/L, 8.2; $p=0.11$) groups. The size of the fall in haemoglobin concentration was not significantly associated with primaquine dose ($p=0.46$). The timing of the nadir in haemoglobin was independent of treatment group, and the greatest contribution to the total decrease in haemoglobin occurred before day 2 when the study drug was administered. By day 28, in all treatment groups, haemoglobin concentrations had recovered and exceeded baseline concentrations (figure 4). We recorded no cases of black water fever; red, black, or tea-coloured urine; or severe haemolysis; and no child needed a blood transfusion. Sex had no effect on safety outcomes (data not shown).

The proportion of participants having adverse events did not differ between treatment groups after adjustment of significance levels for multiple comparisons (data not

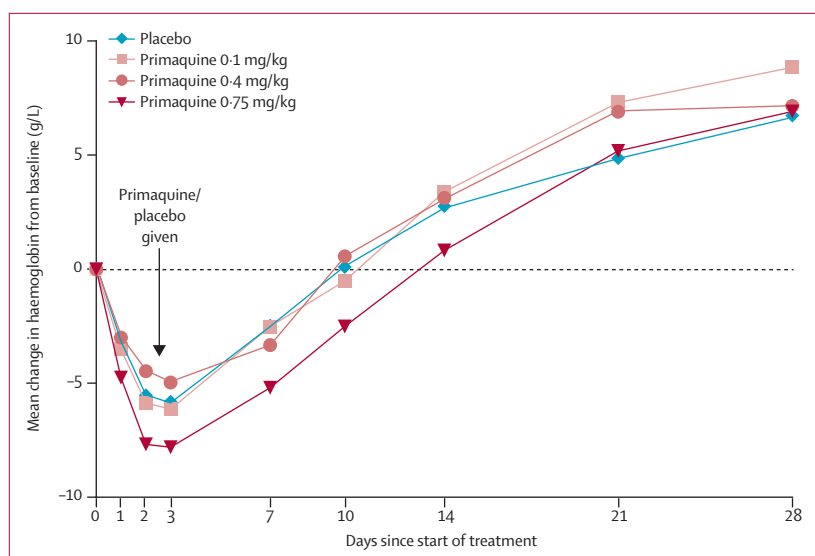


Figure 4: Mean change in haemoglobin measurements by treatment regimen during 28 day follow-up. Haemoglobin concentrations (g/L) during follow-up are expressed relative to that at enrolment for each treatment group.

shown). In the sex-stratified analysis, the maximum reduction in haemoglobin concentration seemed to be larger in the 0.75 mg/kg group compared with the placebo group in girls ($p=0.023$), but this difference was not statistically significant after correction for multiple comparisons (Bonferroni threshold level for significance $p=0.0083$). One child, aged 1.5 years, had a haemoglobin concentration of less than 50 g/L, which was the only severe adverse event. This boy, who received 0.4 mg/kg primaquine, had a baseline haemoglobin concentration of 99 g/L. On day 9 of follow-up, he underwent an elective surgical procedure in a mobile clinic. The mother reported no attempt at haemostasis postoperatively and the child had bled severely. By day 14, his haemoglobin concentration had fallen to 49 g/L without clinical compromise. After wound care and treatment with iron and folate, his haemoglobin concentration recovered to 106 g/L on day 28. This event was judged to be unrelated to the study drug.

Discussion

This study is the first formal dose-finding trial to assess *P falciparum* gametocyte clearance after treatment with single-dose primaquine when given in combination with an ACT (panel). We showed that the duration of gametocyte carriage was roughly halved when 0.75 mg primaquine per kg was given in addition to ACTs. A reduced dose of 0.4 mg/kg had a non-inferior gametocytocidal effect compared with the WHO reference dose, whereas the duration of gametocyte carriage was inconclusive for non-inferiority in the 0.1 mg/kg group and gametocyte prevalence was higher during follow-up than at baseline. Safety outcomes did not differ significantly between the treatment groups.

In this population of children with uncomplicated clinical malaria, gametocytes were detected at baseline in a quarter of children by microscopy compared with four-fifths by molecular methods, which is consistent with previous findings and emphasises the inadequate sensitivity of microscopy in identification of potentially infectious people.³¹ Gametocyte prevalence decreased during follow-up; roughly half of the patients with gametocytes at enrolment cleared their gametocytes during the first 2 days of treatment, before primaquine was given. These dynamics differ from those reported in children in a previous ACT–primaquine trial that showed a more gradual reduction in gametocyte prevalence after ACT,⁷ but are similar to those recorded in symptomatic Kenyan children of the same age group.³ Although primaquine shortened the duration of gametocyte carriage, we noted that even the highest single dose of the drug did not render all participants gametocyte negative. In previous studies in Burma and Indonesia, microscopic gametocytes persisted in a few individuals 21 days after primaquine treatment.^{8,9} In our study, six of 106 (5.7%) children were gametocyte positive by molecular methods on day 14 after initiation of treatment, even with the highest dose of primaquine. However, the density of these persistent gametocytes was much lower than that at enrolment. We used gametocyte density estimates for secondary outcome measures because no clear lower threshold gametocyte density that is needed for successful mosquito infection has been established.^{32–34} The gametocyte circulation time, which was calculated on the basis of the rate of decrease of gametocyte densities after treatment, was significantly longer in the placebo and 0.1 mg/kg groups than in the reference group, but did not differ significantly between the 0.4 mg/kg group and the reference 0.75 mg/kg group. The AUC of gametocyte density over time, a summary measure for malaria transmission potential,^{7,27,35} was numerically higher in the placebo group and 0.1 mg/kg dose group than in the 0.75 mg/kg dose group, but this difference was statistically significant only for the 0.1 mg/kg dose group. There was no significant difference in the AUC between the 0.4 mg/kg and the 0.75 mg/kg dose groups. Baseline differences in asexual parasites between treatment groups did not result in differences in baseline gametocyte prevalence or density or differences in treatment outcome, and did not confound the comparison of gametocyte dynamics between groups.

Although we used sensitive molecular gametocyte detection methods in our trial and therefore provide detail that is absent from most other primaquine trials, a relevant shortcoming of this and other studies is that gametocyte infectiousness to mosquitoes was not established. A proportion of the gametocytes that are observed by microscopy shortly after primaquine treatment might be non-infectious.¹⁵ Whether or not *Pfs25* mRNA can be detected from non-viable gametocytes is unknown, and a proportion of the gametocytes that we detected could have

been non-infectious. We might, therefore, have underestimated the transmission-blocking effect of primaquine. None of the available gametocyte detection devices allow inferences to be made about the infectiousness of gametocytes to mosquitoes, and only mosquito feeding assays can provide definitive evidence for the transmissibility of gametocytes. However, limitations do exist in the extent to which labour-intensive mosquito feeding assays can be used in clinical trials.³⁶ Although gametocyte measurements can be done repeatedly from the same patient, the few clinical trials that have used mosquito feeding assays typically do feeding experiments at one timepoint per participant^{3,37,38} and thereby ignore the dynamics of gametocyte infectivity.³⁸ Future studies that investigate the gametocytocidal effects of low-dose primaquine should therefore preferentially include mosquito feeding assays at intervals during follow-up.

A further limitation of this study was the absence of available paediatric dose formulations for primaquine, which necessitated titration of crushed primaquine in solution for accurate dosing. Although crushed tablets have been used previously for the 0.75 mg/kg dose,^{7,8} this approach might have affected efficacy, especially of the lowest dose (0.1 mg/kg). More data for the relative bioavailability of different formulations of primaquine are needed. Hence, a prerequisite to the scaling up of primaquine deployment will be the availability of reliable paediatric formulations for low doses of the drug.

This study aimed to establish the efficacy and safety of low-dose primaquine in people with normal G6PD enzyme function. G6PD-deficient children were excluded from this study based on the fluorescent spot test, the most widely used enzyme function test¹³ that detects enzyme function to a cutoff of about 20–30% of normal activity.³⁹ We decided to exclude G6PD-deficient children so that we could first establish the lowest efficacious dose before vulnerable patients are exposed to a potentially haemolytic drug. Although haemolysis has been reported in people without common mutations in the G6PD enzyme,²⁹ the exclusion of those with abnormal enzyme function does clearly limit the generalisability of the safety outcomes of this study and this issue needs to be addressed in future studies. Given this caveat, haemoglobin concentrations fell most rapidly in the first 2 days after enrolment in all study groups, which implies that the greatest effect on haemoglobin was caused by clinical malaria rather than a drug effect. Thereafter, haemoglobin recovered to pre-morbid concentrations. A similar trend has been recorded in children in Tanzania,⁷ and in populations in Burma³⁰ and Indonesia.⁹ We recorded no children with objective measures of clinically significant haemolysis or black urine, or who needed hospital admission or blood transfusion. The only severe adverse event was in a child who underwent an elective surgical procedure unrelated to the clinical malaria episode on day 9 and

Panel: Research in context

Systematic review

We searched PubMed on May 25, 2013, without date or language restrictions, with the terms “primaquine” and “malaria, falciparum” and “gametocyte” or “primaquine” and “malaria, falciparum” and “transmission”. We identified no randomised controlled trials assessing the dose-response relation of primaquine for gametocytocidal activity. A Cochrane review of the transmission-reducing efficacy of primaquine published in September, 2012, identified five trials assessing a primaquine-artemisinin combination therapy combination that satisfied the criteria for inclusion and none of these analysed a range of doses.²⁸ Three studies have assessed the haematological safety of primaquine with artemisinin combination therapies,^{7,29,30} but our trial is unique in that it was specifically powered to assess safety outcomes. A search of clinical trial registration sites for primaquine dose-finding trials for transmission blocking showed one trial that is underway in The Gambia (NCT01838902) to assess the efficacy of artemisinin combination therapy alone, and with 0.2 mg/kg, 0.4 mg/kg, or 0.75 mg/kg primaquine base in asymptomatic patients. This trial is scheduled for completion in 2015. Another study (NCT01743820), which is in development, will assess primaquine dose escalation from 0.125 mg/kg in 50 participants randomly allocated to different dosing groups. Several other registered studies with primaquine for *Plasmodium falciparum* do not involve dose-finding but will address relevant questions for the future wide-scale deployment of primaquine. These studies include a trial of the optimum timing of primaquine administration (NCT01906788, recruiting), primaquine pharmacokinetics (NCT01552330 and NCT01525511, both completed August, 2013), and a trial with mosquito feeding as an endpoint that will compare artemisinin combination therapy alone with 0.75 mg/kg primaquine (NCT01849640, not yet recruiting, with a scheduled 3-year timeline).

Interpretation

This study is, to our knowledge, the first randomised, placebo-controlled trial to assess the dose-response relation of one dose of primaquine for gametocyte clearance and for safety in falciparum malaria. This trial was undertaken in African children with clinical malaria and normal glucose-6-phosphate dehydrogenase enzyme function. A dose reduction to 0.4 mg/kg primaquine base had demonstrable non-inferiority to the reference 0.75 mg/kg dose, whereas a dose of 0.1 mg/kg was inconclusive for non-inferiority. This trial was designed and started before a revision of the WHO guidelines recommending 0.25 mg/kg primaquine for transmission blocking, in light of which this new dose must now be assessed. In this population, all doses of primaquine had similar safety profiles to placebo. An study of low-dose primaquine in people with glucose-6-phosphate dehydrogenase deficiency is warranted.

therefore after the expected duration of primaquine-associated haemolysis.

In this dose-finding trial, primaquine administration was delayed until day 2 after initiation of schizonticidal therapy. This timepoint is when, in the context of uncomplicated malaria, the rate of malaria-attributable haemolysis is expected to be falling, and comparisons of haematological effects between dose groups are expected to be less affected by the consequences of acute malaria infection. In operational terms, administration of primaquine on the first day of schizonticidal treatment is probably advantageous, and comparisons of the efficacy of day 0 versus day 2 administration will be important.

For more than 40 years, WHO has recommended a single dose of 0.75 mg primaquine base per kg in

combination with schizonticidal drugs to reduce transmission of malaria.⁴⁰ However, no dose-finding trials underpinned this recommendation. The small evidence base for primaquine use has prompted uncertainty as to the benefit of an intervention that carries a documented risk of haemolysis in malaria-endemic populations.^{28,41} The real threat of spreading artemisinin resistance⁴² has led to urgency in addressing this problem. In September, 2012, while our study was ongoing, an evidence review group commissioned by WHO revised its recommended dose to 0.25 mg primaquine base per kg to be added to ACT to treat parasitologically confirmed falciparum malaria infection in new programmes for malaria elimination and to stop the spread of artemisinin resistance.⁴³ This dose revision was based on underpowered historical studies, and the need for contemporary data was emphasised.⁴⁴ The 0.25 mg/kg dose was not assessed in our study, which is a limitation and leaves important questions to be addressed in future dose-finding trials. However, we have shown that gametocytocidal efficacy is retained when the primaquine dose is reduced from 0.75 mg/kg to 0.4 mg/kg and that a dose-response relation exists for lower doses. The finding of reduced gametocytocidal efficacy at doses lower than 0.4 mg/kg seems to contradict suggestions of uniform efficacy in the range of 0.065–0.75 mg primaquine per kg.¹⁶ This new information provides a valuable starting point for identification of the most efficacious and safest low dose of primaquine for transmission blocking. Subsequent investigations of primaquine should include assessments of the efficacy of doses lower than 0.4 mg/kg (including the newly recommended 0.25 mg/kg dose), with use of mosquito transmission endpoints to allow for differences in infectiousness of gametocytes persisting after treatment; the optimum timing of primaquine in combination with ACT; the pharmacokinetics of low-dose primaquine; and the safety of low-dose primaquine in people with G6PD enzyme deficiency, which is of high priority. Because of differences in gametocyte dynamics between African and Asian settings⁴⁵ and differences in the severity of G6PD deficiency across regions,⁴⁶ studies in a range of malaria-endemic settings are needed.

Contributors

ACE, TB, SY, NJW, ELW, SGS, and CD designed the study and were involved in interpretation and writing. ACE implemented and led the study. JB and ELW provided statistical support for data analysis. AO and GG participated in data collection. LG and KHWL did the QT-NASBA laboratory analysis. MK, HW, AM, and SN provided logistical support. All authors reviewed and approved the final version.

Conflicts of interest

We declare that we have no conflicts of interest.

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