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Research Paper

The Gametocytocidal Efficacy of Different Single Doses of Primaquine with Dihydroartemisinin-piperaquine in Asymptomatic Parasite Carriers in The Gambia: A Randomized Controlled Trial☆

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ABSTRACT

Background: Asymptomatic low-density gametocyte carriers represent the majority of malaria-infected individuals. However, the impact of recommended treatment with single low dose of primaquine and an artemisinin-based combination therapy to reduce transmission in this group is unknown.

Methods: This was a four-arm, open label, randomized controlled trial comparing the effect of dihydroartemisinin-piperaquine (DHAP) alone or combined with single dose of primaquine (PQ) at 0.20 mg/kg, 0.40 mg/kg, or 0.75 mg/kg on *Plasmodium falciparum* gametocytaemia, infectiousness to mosquitoes and hemoglobin change in asymptomatic, malaria-infected, glucose-6-phosphate dehydrogenase (G6PD) normal individuals. Randomization was done using a computer-generated sequence of uneven block sizes with codes concealed in sequentially numbered opaque envelopes. The primary endpoint was the prevalence of *P. falciparum* gametocytemia at day 7 of follow-up determined by quantitative nucleic acid sequence based assay and analysis was by intention to treat. The trial has been concluded (registration number: NCT01838902; https://clinicaltrials.gov/ct2/show/NCT01838902).

Results: A total of 694 asymptomatic, malaria-infected individuals were enrolled. Gametocyte prevalence at day 7 was 37.0% (54/146; 95% CI 29.2–45.4), 19.0% (27/142; 95% CI 12.9–26.4), 17.2% (25/145; 95% CI 11.0–23.5) and 10.6% (15/141; 95% CI 6.1–16.9) in the DHAP alone, 0.20 mg/kg, 0.40 mg/kg, and 0.75 mg/kg PQ arms, respectively. The main adverse events reported include headache (130/471, 27.6%), cough (73/471, 15.5%), history of fever (61/471, 13.0%) and abdominal pain (57/471, 12.1%). There were five serious adverse events however, none was related to the interventions.

Interpretation: A single course of PQ significantly reduces gametocyte carriage in malaria-infected asymptomatic, G6PD-normal individuals without increasing the risk of clinical anemia. The limited number of successful mosquito infections suggests that post-treatment transmission potential in this asymptomatic population is low.

Abbreviations: ACT, artemisinin-based combination therapy; ANOVA, analysis of variance; CI, confidence interval; CYP2D6, cytochrome P450 2D6; DHAP, dihydroartemisinin-piperaquine; G6PD, glucose-6-phosphate dehydrogenase; HR, hazards ratio; IQR, interquartile ratio; MD, mean difference; MRC, Medical Research Council; SD, standard deviation; PQ, primaquine; QT-NASBA, quantitative nucleic acid sequence based assay.

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1. Introduction

Asymptomatic, low-density infections constitute over 60% of the human reservoir of malaria parasite (Laishram et al., 2012) and this combined with long periods of carriage without progression to clinical disease (Bereczky et al., 2004), even in low transmission settings, suggests that asymptomatically infected individuals may contribute substantially to malaria transmission (Alves et al., 2005; Mwesigwa et al., 2015). In contrast, clinical malaria cases have been associated with higher parasite densities. However, the relation between pre-treatment asexual parasite density and gametocyte prevalence after treatment has not been consistent. Antimalarial treatment clears the asexual parasite load which in turn reduces gametocyte burden but clearance of mature gametocytes present prior to treatment is incomplete and varies by treatment (WWARN Gametocyte Study Group, 2016). Therefore, for interrupting malaria transmission and eventual elimination, efficient surveillance and treatment of all persons infected with both asexual stages and gametocytes (Slater et al., 2015) is important.

Primaquine (PQ), an 8-aminoquinoline, is recommended in combination with an artemisinin-based combination therapy (ACT) in low *Plasmodium falciparum* transmission settings to further reduce transmission (World Health Organization, 2010). These drugs act complimentarily: ACTs rapidly clear the *P. falciparum* asexual parasite biomass as well as early gametocyte stages (Chotivanich et al., 2006), considerably reducing post-treatment gametocyte carriage (WWARN Gametocyte Study Group, 2016) while PQ clears mature gametocytes (White, 2008). However, implementation has been slow because PQ causes a dose-dependent hemolysis, particularly in individuals with some deficiency of the red blood cell enzyme, glucose 6-phosphate dehydrogenase (G6PD) (Eziefula et al., 2014b). The mean prevalence of G6PD deficiency variant in sub-Saharan Africa is 7.5% (Nkhoma et al., 2009) but varies significantly by and within country (Howes et al., 2012). Lower PQ doses may reduce the risk of hemolytic events. The recommended dose was recently reduced from 0·75 mg base/kg to 0·25 mg base/kg to minimize this risk of hemolysis (Global Malaria Programme, 2015) while presumably retaining efficacy (Ashley et al., 2014; White et al., 2012). PQ's mode of action is unclear but may act by sterilizing gametocytes and thus preventing fertilization in the mosquito; this effect precedes clearance of gametocytes from circulation (White, 2013). The presence of circulating gametocytes is thus a poor predictor of transmissibility (Karunajeewa and Mueller, 2016). The efficacy of PQ has been measured by gametocyte clearance and infectiousness to mosquitoes. However, infectiousness studies are not well standardized and this affects their suitability for evaluating efficacy of transmission-blocking interventions (Bousema et al., 2012).

Early trials on the 0.75 mg/kg dose reported variable reductions in gametocyte carriage (El-Sayed et al., 2007; Shekalaghe et al., 2011; von Seidlein et al., 2003) and a dose-ranging trial in clinically ill patients showed efficacy at doses as low as 0.40 mg/kg (Eziefula et al., 2014a); however, non-inferiority of the 0·1 mg/kg dose was inconclusive (Eziefula et al., 2014a). More recent studies in high transmission areas and high-density gametocyte carriage confirm that PQ reduces gametocyte carriage and infectiousness to mosquitoes over a range of doses that includes the currently recommended 0.25 mg/kg (Dicko et al., 2016; Goncalves et al., 2016). There are indications that the added value of PQ in reducing post-treatment infectivity may differ between symptomatic and asymptomatic infections (El-Sayed et al., 2007; Shekalaghe et al., 2007) and the dynamics of asymptomatic low-density parasite carriage may be markedly different (WWARN Gametocyte Study Group, 2016), perhaps due to longer periods of infection in asymptomatic infections and hence more circulating mature gametocytes (Price et al., 1999; Stepniewska et al., 2008). Therefore, there is the need for evidence on the efficacy and safety of these PQ doses in asymptomatic individuals with low-density malaria infections and their impact on infectiousness to mosquitoes.

This study compared the gametocytocidal efficacy of three different single doses of PQ combined with dihydroartemisinin-piperaquine (DHAP) on gametocyte carriage in asymptomatic, malaria-infected, G6PD-normal individuals in The Gambia. Infectivity to *Anopheles coluzzii* mosquitoes was measured in a subset of enrolled participants.

2. Method

2.1. Study Design and Participants

This was a 4-arm, parallel open-label randomized controlled trial conducted in the Central and Upper River Regions of The Gambia. The trial protocol with details of the design has been published previously (Okebe et al., 2015). Asymptomatic individuals with *P. falciparum* mono-infection and a parasite density >20 parasites/μl were identified by systematic community-based screening after an informed consent. Additional eligibility criteria included: age >1 year, axillary temperature <37.5 °C and no history of fever in the 24 h before the visit, normal G6PD status determined by fluorescent spot test (N. Dimopoulos SA) and a hemoglobin value ≥8 g/dl (HemoCue, Ängelholm, Sweden). Individuals who reported sickle cell disease, antimalarial use within two weeks prior to screening, current pregnancy or history of allergy to the study drugs were excluded.

2.2. Randomization and Allocation Concealment

All participants received DHAP (Sigma-Tau IFR S.p.A, Italy) and in addition, three of the four arms were randomized to receive a single dose of PQ at 0.75 mg, 0.40 mg or 0.20 mg base/kg body weight using a computer-generated sequence of uneven block sizes. In the first phase of the study (participants 1–347) PQ was procured from the Government Pharmaceutical Organization in Thailand; during the second phase of the study (participants 348–694) PQ was procured from Sanofi®, (Forest Park, GA, USA). The source was changed to harmonize the intervention with other on-going trials with PQ (Eziefula et al., 2012). The randomization codes for the trial arms were concealed in sequentially numbered opaque envelopes and opened by the study clinician to reveal the allocation arm and, based on body weight, calculate the dose for both DHAP and PQ. A separate randomization sequence was used to select a third of those enrolled in the trial for membrane feeding experiments.

2.3. Treatment and Procedures

DHAP was given once daily for 3 days on an empty stomach while PQ was given on the last day of ACT treatment at least one hour after the last dose of DHAP with a light snack. For small children, a tablet of PQ (15 mg base) was crushed and reconstituted in 15 ml of water to produce a 1 mg/ml suspension and the required dose was drawn, to the nearest 0.1 ml, mixed with orange juice and administered. A study nurse directly supervised all treatments and the participant was observed for 30 min for any reaction; if the drug was vomited within this period, it was repeated.

The choice of administration of PQ on day 2 was based on available evidence (Eziefula et al., 2012). ACTs have been shown to sharply reduce gametocyte density with the most pronounced effect within the first three days of administration, after which there is a more gradual decay of persisting gametocytes (Bousema et al., 2010). Therefore, giving PQ on day 2 provides a clearer indication of its gametocyte-clearing effect. Also, administering PQ on day 2 could limit the impact of both the clinical disease and treatment on hemoglobin levels (Eziefula et al., 2014a).

Participants were reviewed on days 1, 2, 3, 7, 10, 14, and then weekly till day 42 with clinical examinations and blood samples collected for hemoglobin check and for quantifying gametocytes marker *Pfs*25 mRNA by quantitative nucleic acid sequence based amplification (QT-