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Safety and efficacy of dihydroartemisinin/piperaquine (Artekin®) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Rwandan children

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Summary In Rwanda, amodiaquine + sulfadoxine/pyrimethamine (AQ + SP) is the current first-line treatment for malaria, introduced in 2001 as an interim strategy before the future deployment of an artemisinin-based combination treatment (ACT). Dihydroartemisinin/piperaquine (DHA-PQP) is a new co-formulated and well tolerated ACT increasingly used in Southeast Asia where it has proved to be highly effective against *Plasmodium falciparum* malaria. We tested the efficacy, safety and tolerability of DHA-PQP in children with uncomplicated *P. falciparum* malaria. A randomised, open trial was carried out in 2003—2004. Seven hundred and sixty-two children aged 12—59 months with uncomplicated *P. falciparum* malaria were randomly allocated to one of the following treatments: amodiaquine + artesunate; AQ + SP; or DHA-PQP. Patients were followed-up until Day 28 after treatment. Adverse events and clinical and parasitological outcomes were recorded. Children treated with DHA-PQP or AQ + AS had a significantly higher cure rate compared with those treated with amodiaquine + sulfadoxine/pyrimethamine (95.2% and 92.0% vs. 84.7%, respectively). Parasite clearance was significantly faster in children treated with DHA-PQP and AQ + AS compared with those treated with amodiaquine + sulfadoxine/pyrimethamine. The frequency of adverse events was significantly lower in patients treated with DHA-PQP than in those treated with

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combinations containing amodiaquine. A 3-day treatment with DHA-PQP proved to be efficacious with a good safety and tolerability profile and could be a good candidate for the next first-line treatment.

1. Introduction

In 2001, following high levels of chloroquine (CQ) resistance in Rwanda, the combination amodiaquine + sulfadoxine/pyrimethamine (AQ + SP) was adopted as the first-line antimalaria treatment. Since then its efficacy has steadily declined. In 2002 the adequate clinical and parasitological response (ACPR) at Day 28 was estimated as 83% (Rwagacondo et al., 2003), in 2003 it was 74% (present study) and in 2004 it was 64% (Fanello et al., unpublished data). Moreover, its implementation at the national level has been compromised by low tolerability, with a substantial proportion of adult patients experiencing pruritus and fatigue (Fanello et al., 2006). AQ + SP has always been considered an interim strategy and different artemisinin-based combination treatments (ACT) have been tested in the past few years as possible alternatives.

Dihydroartemisinin/piperaquine (DHA-PQP), also known under the brand name Artekin®, is a new artemisinin-containing fixed-combination antimalarial treatment that has proved to be well tolerated and highly effective against *Plasmodium falciparum* malaria in Southeast Asia (Ashley et al., 2004, 2005; Denis et al., 2002). Piperaquine, an orally active bisquinoline, is structurally related to CQ. It is likely that piperaquine and aminoquinolines such as CQ have similar targets, possibly inhibition of the heme digestion pathway in the parasite food vacuole (Davis et al., 2005). Some resistance did develop in China when piperaquine was used widely as monotherapy against CQ-resistant *P. falciparum*. However, in combination with dihydroartemisinin it was shown to be effective in areas of multidrug-resistant *P. falciparum* (Ashley et al., 2004; Tran et al., 2004).

In the present clinical trial, we compared the safety, tolerability and efficacy of DHA-PQP with that of AQ + SP and amodiaquine + artesunate (AQ + AS) at three Rwandan sites.

2. Materials and methods

2.1. Study design, sites and patient treatment

This was a randomised, open trial carried out at three sites in Rwanda: Kicukiro, Mashesha and Rukara. Kicukiro is an urban/peri-urban health centre near Kigali. Rukara and Mashesha are both rural health centres; the first is located near the eastern border with Uganda and the second near the border with the Democratic Republic of Congo, at 900 m above sea level in a rice cultivation area. Patients attending the health centres with suspected clinical malaria were screened and enrolled in the study if they met the following inclusion criteria: age 12—59 months; weight $\geq$10 kg; monoinfection with *P. falciparum*; parasite density 2000—200 000/μl; fever (axillary body temperature $\geq$37.5 °C) or history of fever in the preceding 24 h; and packed cell volume (PCV) >15%. Patients with severe malaria, mixed malaria infection, any other concomitant illness or underlying disease, known allergy to the drugs being used in this trial, or a clear history of adequate antimalarial treatment in the previous 72 h were excluded. Cases of severe malaria were referred to the nearest hospital for treatment with intravenous quinine and other supportive therapy. Patients with mixed infections were treated according to national guidelines.

Patients were randomly allocated in blocks of 15 to receive one of the three treatments: DHA-PQP, AQ + SP or AQ + AS. Treatments were administered according to body weight. AQ + AS: AQ 10 mg/kg/day and AS 4 mg/kg/day (Arsumax®; Sanofi, Gentilly, France) for 3 days; AQ + SP: AQ 10 mg/kg/day for 3 days and SP 25 mg/kg of sulfadoxine and 1.25 mg/kg of pyrimethamine the first day; DHA-PQP: DHA 1.6—3.1 mg/kg/day and PQP 12.8—24.6 mg/kg/day for 3 days (one tablet contains 40 mg of DHA and 320 mg of PQP; Artekin®; Holleypharm, China).

After drug administration, patients were observed for 1 h and the dose was repeated in full if vomiting occurred within 30 min or halved if vomiting occurred between 30 min and 1 h post dosing. If vomiting persisted, the patient was hospitalised and rescue treatment was given. A case record form was completed for each patient documenting all symptoms prior to clinic attendance, concomitant illness and drug history.

All subjects enrolled in the study were given a unique code corresponding to the randomisation list and received the corresponding treatment. Allocation of treatment was concealed until final recruitment of the patient.

2.2. Follow-up

Parents/guardians of children were asked to return 24 h and 48 h later for drug administration as well as for scheduled tests at 72 h and 7, 14, 21 and 28 days. If the patient did not report for scheduled visits, every effort was made to locate him or her at the home address. Parents/guardians were encouraged to take the child to the hospital whenever the child was sick. At each visit, the history, clinical signs and symptoms, body temperature and a blood sample for parasitaemia were collected. A blood spot on filter paper for molecular analysis was also collected at each visit.

2.3. Laboratory methods

Thick blood films were stained with Giemsa. Parasite density was determined on the basis of the number of parasites per 200 white blood cells (WBC) on a thick film, assuming a total WBC count of 8000/μl. If gametocytes were seen, the gametocyte count was extended to 1000 WBCs. Laboratory technicians reading malaria slides did not know the treatment received by individual patients. PCV (measured by microhaematocrit centrifugation) as well as total and

differential WBC counts were assessed at Days 0 and 14 for all patients, and liver function tests, aspartate aminotransferase and alanine aminotransferase were assessed at Days 0 and 14 for patients attending Kicukiro health centre. If the child had a second episode of parasitaemia between Days 15 and 28, blood samples on filter paper from the first and second episodes were used to type parasite strains to distinguish between new infections and recrudescence. DNA was purified (Irion et al., 1998) and two polymorphic markers of *P. falciparum* (the three sequence families of the MSP1 block 2 repeat region and the two sequence families of the MSP2 repeat region) were analysed (Snounou and Singh, 2002). A recrudescent infection was defined as one that matched in size at least one allele of both the MSP1 and MSP2 loci between the first and second samples. If any clone of a polyclonal primary infection was detected during a second episode, this was considered a recrudescence.

2.4. Outcome measurements

The primary outcome measure in this study was the incidence of microscopically and genotypically confirmed recrudescent infections in the different treatment groups by Day 28. Secondary measures were the immediate treatment responses: parasite and fever clearance and occurrence of adverse events (AE). Treatment outcome was established according to standard WHO classification (WHO, 2003). Early treatment failure (ETF) was defined as: (i) danger signs or severe malaria on Days 1, 2 or 3 with parasitaemia; (ii) parasite density at Day 2 greater than at Day 0; (iii) parasitaemia on Day 3 with axillary temperature $\geq$37.5 °C; and (iv) parasite density at Day 3 equal to or greater than 25% that at Day 0. Late clinical failure (LCF) was defined as danger signs, severe malaria or parasitaemia with axillary temperature $\geq$37.5 °C between Days 4 and 28 without having been previously classified as ETF. Late parasitological failure (LPF) was defined as the reappearance of parasitaemia between Day 4 and Day 28 without fever and without previously meeting any of the criteria for ETF or LCF. ACPR was defined as the absence of parasitaemia by Day 28 without previously meeting any of the criteria for ETF, LCF and LPF. The number of cases of total treatment failure was computed as ETF + LCF + LPF. A full course of quinine was administered as rescue treatment according to Rwandan National Treatment Guidelines to all patients considered as treatment failures.

All AEs were recorded on the clinical record form. An AE was defined as 'any unfavourable and unintended sign, symptom, or disease temporally associated with the use of the drug administered'. A causality assessment of the AEs was done according to the guidelines of WHO-Uppsala Monitoring Centre.

2.5. Statistical analysis

Data were double entered and validated using Epi Info 6.4b (CDC, Atlanta, GA, USA). Descriptive statistics were used to summarise baseline values and demographic data. For the per protocol analysis, χ^2 was used to compare proportions. The odds ratio (OR) for failure was calculated with 95% CI with a two-sided Fisher's test. Mantel–Haenszel (MH) was used to adjust for sites. For the intention-to-treat analysis, the log-rank test and the hazard ratio (Cox regression) adjusted by site were estimated. ANOVA was used for normally distributed continuous data. The non-parametric Kruskal–Wallis test was used to analyse continuous data with a skewed distribution. All analyses were performed using STATA statistical analysis software package version 8 (Stata Corp., College Station, TX, USA).

2.6. Ethical approval

The study was reviewed and approved by the Ministry of Health of Rwanda and by the Ethical Committee of the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. Informed written consent was provided by the parents/guardians of all patients before inclusion in the study.

3. Results

Between October 2003 and April 2004, 762 patients were recruited in the three study sites (AQ + AS = 252, AQ + SP = 258 and DHA-PQP = 252). At enrolment, the groups had similar demographic and clinical characteristics (Table 1). Four patients were lost to follow-up: one in the AQ + AS group, one in the AQ + SP group and two in the DHA-PQP group. Two patients in the AQ + SP group were excluded because they were treated with co-trimoxazole during follow-up (Figure 1).

The proportion of patients still parasitaemic by Day 2 was significantly lower in the DHA-PQP group (6.0%; 15/251) than in the AQ + AS group (11.2%; 28/251) ($P = 0.04$), and the latter was significantly lower than the AQ + SP group (35.5%; 91/256) ($P = 0.0001$). The proportion of patients parasitaemic by Day 3 was significantly lower both in the DHA-PQP (0.4%; 1/251) and the AQ + AS (0.8%; 2/249) groups than in the AQ + SP group (5.1%; 13/255) ($P < 0.0001$). No differences were observed among groups in fever clearance at any day.

ETFs were observed in all treatment groups: three patients in the AQ + AS group, five patients in the AQ + SP group and one patient in the DHA-PQP group.

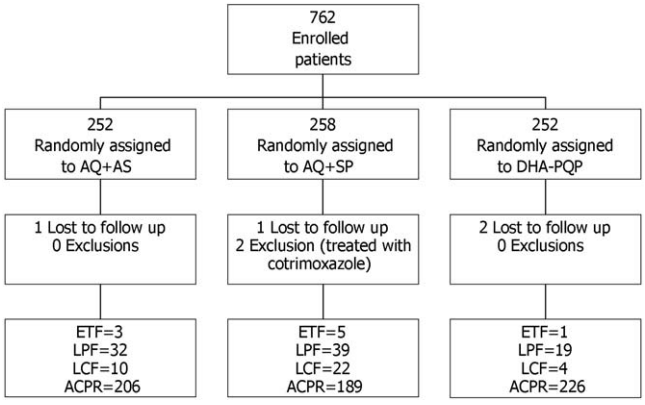

Figure 1 Trial profile. AQ + AS: amodiaquine + artesunate; AQ + SP: amodiaquine + sulfadoxine/pyrimethamine; DHA-PQP: dihydroartemisinin/piperaquine; ETF: early treatment failure; LPF: late parasitological failure; LCF: late clinical failure; ACPR: adequate clinical and parasitological response.

Table 1 Baseline characteristics of the patients according to treatment

	AQ + AS (*N* = 252)	AQ + SP (*N* = 258)	DHA-PQP (*N* = 252)
Demography			
Female:male	117:135	113:145	131:121
Mean age (months) (SD)	34.3 (15.0)	34.2 (14.1)	36.3 (14.4)
Weight (kg) (SD)	12.6 (2.8)	12.6 (2.7)	12.9 (2.5)
Clinical characteristics			
Mean temperature (°C) (SD)	38.3 (1.2)	38.3 (1.3)	38.4 (1.3)
Geometric mean asexual *Plasmodium falciparum*/μl (95% CI)	31952 (27355—37321)	28355 (24370—32991)	29999 (25735—34970)
Gametocyte rate (%)	5.6 (14/252)	2.7 (7/258)	3.2 (8/252)
Splenomegaly (%)	6.0 (15/252)	5.8 (15/258)	10.0 (25/251)
Hepatomegaly (%)	0.0 (0/251)	0.4 (1/258)	0.4 (1/252)
Laboratory			
PCV (SD)	31.0 (5.5)	31.5 (5.3)	31.5 (4.9)

AQ + AS: amodiaquine + artesunate; AQ + SP: amodiaquine + sulfadoxine/pyrimethamine; DHA-PQP: dihydroartemisinin/piperaquine; PCV: packed cell volume.

Table 2 Clinical and parasitological failure up to Day 28 after enrolment

Site	Treatment outcome	AQ + AS	AQ + SP	DHA-PQP
Mashesha	*N*	89	93	87
	ETF	0	1 (1.1)	0
	LCF	4 (4.5)	6 (6.5)	0
	LPF	9 (10.1)	10 (10.8)	3 (3.4)
	TTF	13 (14.6)	17 (18.3)	3 (3.4)
	TTF (PCR-corrected)	4 (4.5)	10 (10.8)	1 (1.1)
Kicukiro	*N*	74	72	75
	ETF	3 (4.1)	3 (4.2)	0
	LCF	1 (1.4)	3 (4.2)	0
	LPF	1 (1.4)	10 (13.9)	3 (4.0)
	TTF	5 (6.8)	16 (22.2)	3 (4.0)
	TTF (PCR-corrected)	4 (5.4)	11 (15.3)	1 (1.3)
Rukara	*N*	88	90	88
	ETF	0	1 (1.1)	1 (1.1)
	LCF	5 (5.7)	13 (14.4)	4 (4.5)
	LPF	22 (25.0)	19 (21.1)	13 (14.8)
	TTF	27 (30.7)	33 (36.7)	18 (20.5)
	TTF (PCR-corrected)	12 (13.6)	18 (20.0)	10 (11.4)
All sites	ETF	3 (1.2)	5 (2.0)	1 (0.4)
	LCF	10 (4.0)	22 (8.6)	4 (1.6)
	LPF	32 (12.7)	39 (15.3)	19 (7.6)
	TTF	45 (17.9)	66 (25.9)	24 (9.6)
	ACPR	206 (82.1)	189 (74.1)	226 (90.4)
PCR-corrected	LCF	2 (0.8)	10 (3.9)	1 (0.4)
	LPF	15 (6.0)	24 (7.4)	10 (4.0)
	TTF	20 (8.0)	39 (15.3)	12 (4.8)
	ACPR	231 (92.0)	216 (84.7)	238 (95.2)

AQ + AS: amodiaquine + artesunate; AQ + SP: amodiaquine + sulfadoxine/pyrimethamine; DHA-PQP: dihydroartemisinin/piperaquine; ETF: early treatment failure; LCF: late clinical failure; LPF: late parasitological failure; TTF: total treatment failure; ACPR: adequate clinical and parasitological response.

PCR genotyping was carried out on clinical/parasitological failures between Days 15 and 28 and showed: for AQ + AS, 25 new infections, 13 recrudescences and 4 indeterminate results; for AQ + SP, 27 new infections, 24 recrudescent infections and 1 indeterminate result; and for DHA-PQP, 12 new infections, 7 recrudescences and 2 indeterminate results; indeterminate results were considered as failures. The PCR-adjusted ACPR at Day 28 was lower in the AQ + SP group (84.7%; 216/255) than in the DHA-PQP (95.2%; 238/250) and AQ + AS (92.0%; 231/251) groups (Table 2). Taking into account the differences observed among sites, DHA-PQP and AQ + AS ACPRs were not significantly different (MH OR = 0.57; 95% CI 0.27—1.21) ($P = 0.14$); however, the AQ + SP ACPR was significantly lower than that for DHA-PQP (MH OR = 0.27; 95% CI 0.13—0.54) ($P < 0.0001$) and for AQ + AS (MH OR = 0.47; 95% CI 0.27—0.85) ($P = 0.01$). The log-rank test and Cox regression, both adjusted by site, gave identical results to the per protocol analysis.

No differences in gametocyte prevalence were observed between groups.

At recruitment, mean PCV was similar in the three treatment groups. By Day 14, the mean PCV had increased in all groups, although it was lower in the DHA-PQP group (mean PCV = 33.4, SD 3.6) than in the AQ + AS (mean PCV = 34.0, SD 3.7) ($P = 0.08$) and AQ + SP (mean PCV = 34.5, SD 3.7) groups ($P = 0.001$) (data adjusted for differences among sites).

The mean WBC count at Day 14 was similar in all groups. In all treatment groups at Day 14 the proportion of patients with neutropenia (neutrophil count <1000/μl) had increased from Day 0 (AQ + AS: 14.2% (35/247); AQ + SP: 13.5% (34/252); DHA-PQP: 8.4% (21/249)), with no significant differences between groups (Table 3).

No hepatotoxicity was observed, although analyses were performed at one site only (data not shown).

One-hundred and thirty patients reported at least one AE concomitant with the administration of the study drug (68 of them had two or more AEs), 47 (18.65%) patients in the AQ + AS group, 54 (20.93%) in the AQ + SP group and 29 (11.51%) patients with DHA-PQP ($P = 0.01$). Overall, 227 AEs classified as possibly or probably/likely to be related to the study drug or for which the causality was unknown were observed (Table 4). Fatigue, anorexia and vomiting were the most common AEs and were significantly more frequent in the AQ + AS and AQ + SP groups compared with the DHA-PQP group (fatigue, $P = 0.001$; anorexia, $P = 0.005$; vomiting, $P = 0.007$), with no differences between the AQ + AS and AQ + SP groups.

Table 4 Adverse events by treatment group (two or more adverse events in the same patient are considered separately) (% calculated on the total number patients enrolled)

Adverse event	AQ + AS	AQ + SP	DHA-PQP
Abdominal pain	6 (2.4)	8 (3.1)	6 (2.4)
Anaemia	2 (0.8)	3 (1.2)	0
Angina	0	1 (0.4)	0
Anorexia	13 (5.2)	19 (7.4)	3 (1.2)
Cough	10 (4.0)	8 (3.1)	12 (4.8)
Diarrhoea	1 (0.4)	0	8 (3.2)
Dizziness	0	1 (0.4)	1 (0.4)
Drowsiness	1 (0.4)	0	0
Epistaxis	0	1 (0.4)	0
Eye irritation	2 (0.8)	0	0
Fatigue	12 (4.8)	22 (8.5)	3 (1.2)
Fever	0	0	1 (0.4)
Headache	1 (0.4)	1 (0.4)	2 (0.8)
Haematuria	1 (0.4)	0	0
Jaundice	0	1 (0.4)	0
Lymphadenitis	1 (0.4)	1 (0.4)	0
Nausea	9 (3.6)	9 (3.5)	2 (0.8)
Oedema	1 (0.4)	2 (0.8)	1 (0.4)
Seizures	1 (0.4)	1 (0.4)	0
Skin rashes	2 (0.8)	4 (1.6)	0
Vomiting	17 (6.7)	21 (8.1)	5 (2.0)
Total	80	103	44

AQ + AS: amodiaquine + artesunate; AQ + SP: amodiaquine + sulfadoxine/pyrimethamine; DHA-PQP: dihydroartemisinin/piperaquine.

4. Discussion

DHA-PQP was highly efficacious in treating *P. falciparum* infections in children, with a cure rate of 95.2%, ranging from approximately 99.0% in Mashesha and Kicukiro to 87.0% in Rukara. The combination AQ + AS performed equally well, but AQ + SP (the recommended first-line treatment in Rwanda) was significantly less efficacious than the other two treatments, although cases of ETF were observed in all groups. The three treatment regimens were not equally effective in all sites; in Rukara, the ACPR was much lower than in Kicukiro and Mashesha. This result was not unexpected, as a lower efficacy of other antimalarial treatments had already been observed in this particular site

Table 3 Median white blood cell (WBC) count and differential count (/μl) at Days 0 and 14 by treatment group

	AQ + AS		AQ + SP		DHA-PQP	
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
N	252	247	258	252	252	249
WBC count	4700	4600	4800	4590	4815	4600
Range	1955—17350	2100—13950	1800—13800	1800—16300	2200—13850	2200—13250
Neutrophils	2472	1728	2349	1696	2560	1892
Range	460—11371	464—7602	260—12105	540—8208	396—9389	550—6890
Lymphocytes	2029	2639	2019	2595	2074	2556
Range	403—8502	864—7810	510—7728	1000—10106	352—8694	860—7739

AQ + AS: amodiaquine + artesunate; AQ + SP: amodiaquine + sulfadoxine/pyrimethamine; DHA-PQP: dihydroartemisinin/piperaquine.

(Rwagacondo et al., 2004). The reasons for this are unclear and require further research. The higher proportion of DHA-PQP failures in Rukara could be linked to the high CQ resistance in this site. There are conflicting data on the cross-resistance between piperaquine and other antimalarials, and more particularly CQ (Basco and Ringwald, 2003; Davis et al., 2005). Basco and Ringwald (2003) have recently shown that piperaquine was equally active in vitro against CQ-sensitive and -resistant isolates from Africa. However, in southern China, where CQ-resistant *P. falciparum* is highly prevalent and where sensitivity to piperaquine has been monitored since the 1980s, in vitro tests showed a general decline in susceptibility (Fan et al., 1998; Yang et al., 1992, 1995, 1999) along with a reduced clinical efficacy (Guo, 1993). Nevertheless, DHA-PQP was found to be highly efficacious in areas of multidrug resistance (Ashley et al., 2004; Denis et al., 2002; Tran et al., 2004).

DHA-PQP was better tolerated than the other two drugs (although the study was open and therefore the attribution of AEs was to some extent subjective), with significantly fewer AEs. This is possibly owing to the low tolerability of amodiaquine itself, a phenomenon already observed in Rwanda in adult patients (Fanello et al., 2006). The similar occurrence of AEs in the AQ + SP and AQ + AS groups, mainly fatigue, anorexia and vomiting, supports this hypothesis and such low tolerability could compromise the deployment of an ACT as a first-line treatment.

DHA-PQP is relatively inexpensive (approximately US$1.5—3.0 for an adult treatment), given once a day for 3 days and well tolerated. Whilst already licensed in some Asian countries, pharmacokinetic, efficacy and safety data from conventional Phase I—IV trials have not been systematically collected. A recent study on the pharmacokinetic characteristics of DHA-PQP (Hung et al., 2004) showed that piperaquine is highly lipid soluble with an estimated median elimination half-life ($t_{1/2,z}$) of 23 days (19—28 days) in adults and 14 days (10—18 days) in children, thus confirming previous data showing a terminal half-life of 17.3 days in plasma (Hung et al., 2003). The other two combinations tested in the present study contained amodiaquine, which is a pro-drug for the active antimalarial metabolite desethylamodiaquine with an estimated elimination half-life of 1—3 weeks (Krishna and White, 1996); whereas for pyrimethamine and sulfadoxine the half-life is assumed to be 95.5 h and 184 h, respectively (Weidekamm et al., 1982). In theory, combinations of artemisinin derivatives, which are eliminated very rapidly, with a slowly eliminated drug such as piperaquine, would 'protect' the artemisinin derivatives from selection of resistant mutants. However, the slowly eliminated 'tail' of piperaquine would provide a selective filter for resistant parasites (Basco and Ringwald, 2003). In Zanzibar, after exposure to the ACT Coartem® (artemether/lumefantrine), a significant increase in the mutation *pfmdr1* 86N was observed, and the authors suggested that this ACT is not robust enough to avoid selection of resistance-associated mutations in some malarial settings (Sisowath et al., 2005).

There is an urgent need for efficacious affordable antimalarial therapies. In this study we showed that 3-day treatment with DHA-PQP is efficacious for the treatment of *P. falciparum* infections and has a good safety and tolerability profile, confirming previous studies from areas of multidrug-resistant malaria in Southeast Asia. However, piperaquine is not presently manufactured to Good Manufacturing Practice standards and no piperaquine-containing preparations are registered with the US Food and Drug Administration or similar international regulatory bodies (Davis et al., 2005). Moreover, further studies are urgently needed to establish the potential cross-resistance between CQ and piperaquine in Africa.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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