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Comparative Efficacy of Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine for the Treatment of Uncomplicated Malaria in Ugandan Children

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Background. In Uganda, artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DHA-PQ) showed excellent treatment efficacy for uncomplicated malaria in prior trials. Because the frequency of resistance to artemisinins and piperaquine is increasing in Southeast Asia and the prevalence of *Plasmodium falciparum* polymorphisms associated with resistance has changed, we reassessed treatment efficacies at 3 sites in Uganda.

Methods. For this randomized, single-blinded clinical trial, children aged 6–59 months with uncomplicated falciparum malaria were assigned treatment with AL or DHA-PQ and followed for 42 days. Primary end points were risks of recurrent parasitemia, either unadjusted or adjusted to distinguish recrudescence from new infection. We assessed selection by study regimens of relevant *P. falciparum* genetic polymorphisms associated with drug resistance.

Results. Of 599 patients enrolled, 578 completed follow-up. There were no early treatment failures. The risk of recurrent parasitemia was lower with DHA-PQ as compared to AL at all 3 sites at 42 days (26.0% vs 47.0%; $P < .001$). Recrudescent infections were uncommon in both the DHA-PQ and AL arms (1.1% and 2.2%, respectively; $P = .25$). Neither regimen selected for *pfcrt* or *pfmdr1* polymorphisms associated with drug resistance.

Conclusions. AL and DHA-PQ remain effective for the treatment of malaria in Uganda. Neither regimen selected for genetic polymorphisms associated with drug resistance.

Clinical Trials Registration. ISRCTN15793046.

Keywords. Treatment efficacy; malaria; artemether-lumefantrine; dihydroartemisinin-piperaquine; Uganda.

Artemisinin-based combination therapy (ACT) is the treatment of choice for uncomplicated malaria in sub-Saharan Africa. In Uganda, artemether-lumefantrine (AL) has been first-line therapy for uncomplicated malaria since 2004, with artesunate-amodiaquine (AS/AQ) as an alternative first-line treatment and dihydroartemisinin-piperaquine (DHA-PQ) as a second-line treatment. A number of ACTs, including AL and DHA-PQ, have shown excellent treatment efficacy in Africa, with genotype-adjusted efficacies generally >95% across the continent [1, 2]. Compared with AL, DHA-PQ decreased the risk of new infections after treatment, owing to an extended posttreatment prophylactic effect [1]. However, the first-line treatments for uncomplicated malaria across Africa are generally AL or AS/AQ, with DHA-PQ approved for use but administered as second-line treatment in many countries [3].

With widespread implementation of AL and decreased use of chloroquine to treat uncomplicated malaria, changes in *Plasmodium falciparum* genetic polymorphisms associated with antimalarial drug susceptibility have been seen in Uganda, as in other African countries [4–7]. In particular, the prevalences of mutations associated with a decreased response to chloroquine, notably *pfcrt* 76T and *pfmdr1* 86Y and 1246Y, have decreased in Uganda [7–9]. Wild-type sequences at these same alleles are selected by use of AL [10–12] and associated with modest decreases in lumefantrine susceptibility [8]. In the context of these changes, a recent trial in Uganda reported that, compared with AL, AS/AQ was associated with significantly decreased risks of recurrent or recrudescent infection after treatment for uncomplicated malaria [13]. These results suggest modest changes in the relative antimalarial efficacies of these ACTs due to changes in parasite genetics.

While the antimalarial efficacies of leading ACTs appear to remain excellent in Africa, there has been concerning emergence of drug resistance in Southeast Asia. First, delayed parasite clearance after treatment with artemisinins, mediated principally by mutations in the kelch13 (K13) gene, was seen in the Greater

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Mekong Subregion [14, 15]. Second, decreased efficacy of piperaquine, mediated by increased copy number of plasmepsin genes, has emerged in the same region [16]. With decreased efficacy of both components of the combination, DHA-PQ is failing in Cambodia, with high rates of recrudescence after treatment for uncomplicated malaria [15]. With worrisome resistance in other areas but potential benefits of DHA-PQ over other ACTs, it is important to determine whether the antimalarial treatment efficacy of this regimen in Uganda has changed. We thus compared the efficacies of AL and DHA-PQ for the treatment of uncomplicated malaria at 3 sites in Uganda. We also evaluated the impacts of the 2 regimens on the selection of genetic polymorphisms associated with drug resistance.

METHODS

Study Design

This was a randomized, single-blinded, controlled trial conducted at 3 health centers in Uganda: Arua Hospital, Arua District, in northwestern Uganda; Amuru Health Centre, Gulu District, in north-central Uganda; and Mbale Hospital, Mbale District, in eastern Uganda. Each site experiences perennial malaria transmission with high transmission intensity [17]. Enrollment criteria were as follows: (1) no previous enrollment in the study; (2) age 6–59 months; (3) weight ≥5 kg; (4) absence of severe malnutrition, defined as a *z* score less than −3, symmetric edema, or mid-upper arm circumference <110 mm; (5) fever, defined as an axillary temperature ≥37.5°C, or history of fever in the prior 24 hours; (6) no history of serious adverse effects or hypersensitivity reactions to study medications; (7) no use of medications that could interact with the study medications; (8) no evidence or danger signs of severe malaria [18]; (9) no evidence of concomitant febrile illness; (10) provision of informed consent by a parent or guardian and agreement to follow-up for 42 days; (11) absence of repeated vomiting with first dose of study medication; and (12) *P. falciparum* monoinfection detected by microscopy, with a parasite density of 2000–200 000 parasites/μL of blood. Laboratory results were not available until after enrollment; patients could be excluded following enrollment, based on test results.

Ethical Considerations

The study protocol was approved by the Makerere University School of Public Health Research and Ethics Committee, the Uganda National Council of Science and Technology, and the World Health Organization Research Ethics Committee. The study is registered at the ISRCTN registry (identifier ISRCTN15793046; available at: http://www.isrctn.com/ISRCTN15793046.

Randomization

A computer-generated randomization list was created for each of the study sites by an individual not involved in the study. Sequentially numbered, sealed, opaque envelopes containing the treatment assignment were prepared and secured in a locked cabinet accessible to the study nurse. The study nurse, who was unblinded, dispensed the assigned medications but was not involved in evaluation of participants. Patients and providers other than the study nurse were not informed of treatment assignments. A placebo tablet was used for the evening dose among children enrolled in the DHA-PQ arm, to simulate AL dosing, although the placebo was not matched to the appearance of AL.

Interventions

The study nurse directly observed all doses of study medications. AL (Coartem [Novaritis]; 20 mg artemether/120 mg lumefantrine) and DHA-PQ (D-Artepp [Guillin]; 40 mg dihydroartemisinin/320 mg piperaquine) were dosed on the basis of weight band as recommended by the World Health Organization [19]. If a dose was vomited within 30 minutes of administration, it was readministered. Children with fever were given paracetamol, and those with a hemoglobin level <10 g/dL were treated with ferrous sulfate and anthelminthics per Integrated Management of Childhood Illness guidelines [20].

After the initiation of therapy, participants were monitored clinically and underwent a finger prick for collection of a blood specimen for malaria parasite detection by blood smear every 6 hours on days 0, 1, and 2 or until smear findings were negative. Additional study visits occurred on days 3, 7, 14, 21, 28, 35, and 42, as well as on any other day when a participant was ill. At study visits, the child underwent a history and physical examination, and a blood sample was collected by finger prick for thick smear and storage on filter paper. The hemoglobin level was measured at day 0 and, when possible, day 42.

Treatment failures were treated with quinine tablets (10 mg/kg) every 8 hours for 5 days. Patients with evidence of severe malaria or danger signs (including hemoglobin <5 g/dL, convulsions, lethargy, inability to drink or breast feed, repeated vomiting, and inability to sit/stand because of weakness) were referred for treatment with parenteral artesunate or quinine [18]. Participants who took antimalarial medications outside the study, experienced adverse events requiring a change in treatment, withdrew informed consent, or were lost to follow-up were censored at the time of these events.

Laboratory Procedures

Parasitemia was diagnosed using thick smears stained for 10 minutes with 10% Giemsa. Follow-up thick and thin smears were stained with 2% Giemsa for 30 minutes. Experienced microscopists were blinded to clinical information and read all slides to determine the parasite species and density, quantified as parasites per 200 leukocytes [21]. All slides were read by 2 microscopists, with disagreements settled by a third microscopist. A third microscopist was needed to read <4% of samples during this study. The hemoglobin level was measured with a portable spectrophotometer (Hemocue).

Parasite DNA was extracted using Chelex. Paired samples collected during enrollment and the time of recurrent infection were genotyped by evaluating amplified *msp-1*, *msp-2*, and up to 4 microsatellites (TA40, TA60, TA81, TA87, or PfPK2) by capillary electrophoresis in a stepwise manner, as previously described [22]. A recrudescent infection was identified if the initial and recurrent samples shared at least 1 allele at every locus. If one of the loci did not match, the infection was classified as a new infection. When amplification of *msp1*, *msp2,* and the microsatellites was unsuccessful, the *Plasmodium* species was determined by nested polymerase chain reaction with gel electrophoresis, as previously described [23]. *P. falciparum* genetic polymorphisms at *pfmdr1* N86Y, Y184F, and D1246Y and *pfcrt* K76T were genotyped using a ligase detection reaction fluorescent microsphere assay, as previously described [24].

Outcomes

The primary outcome was parasitemia, assessed by microscopy within 42 days of treatment, either unadjusted or adjusted to distinguish recrudescence from new infection. Outcomes were classified according to World Health Organization guidelines and included early treatment failure (danger signs, complicated malaria, or failure to adequately respond to therapy on days 0–3), late clinical failure (danger signs, complicated malaria, or fever and parasitemia on days 4–42), late parasitologic failure (asymptomatic parasitemia on days 7–42), and adequate clinical and parasitological response (absence of parasitemia through follow-up) [18]. Secondary outcomes included prevalence of fever on days 1–3, prevalence of parasitemia on days 1–3, parasite clearance half-life determined using the Worldwide Antimalarial Resistance Network parasite clearance estimator [25, 26], change in hemoglobin level between day 0 and day 42 among children who did not develop recurrent parasitemia, prevalence of gametocytes during follow-up, risk of adverse events, and selection of genetic polymorphisms associated with drug resistance.

Adverse events were evaluated at each study visit, graded according to WHO and National Institutes of Health scales and defined on the basis of International Conference on Harmonization guidelines as untoward medical occurrences, and serious adverse events included death, life-threatening experience, hospitalization, incapacity, or events that required medical or surgical intervention to prevent serious outcomes.

Sample Size

Considering a baseline risk of recurrent parasitemia after treatment with AL of 53% and assuming a 90% follow-up rate, a sample size of 100 patients per arm per site was needed to detect a 21% difference in risk of recurrent parasitemia between treatment arms with 80% power and a 2-sided type I error of 0.05.

Statistical Methods

Data were double entered into Microsoft Access and analyzed using Stata, version 14.2 (Stata), and R Studio, version 1.0.143. Efficacy and safety data were evaluated using a modified intention-to-treat analysis that included all participants who met enrollment criteria and completed all follow-up visits up to the time of exclusion. Participants who were enrolled but then excluded on the basis of initial laboratory results were excluded from the analysis. Cumulative risk of recurrent parasitemia was estimated using the Kaplan-Meier product limit formula. Outcomes adjusted by genotyping were censored for loss to follow-up, new infections, or failure of genotyping. Comparisons of cumulative risks of recurrent parasitemia were made using the nonparametric log-rank test. Categorical variables were compared using χ^2 analysis or the Fisher exact test, and continuous variables were compared using the independent-sample *t* test and Wilcoxon rank sum test. *P* values were 2 sided without adjustment for multiple testing, with statistical significance defined as a P value < .05.

RESULTS

Trial Subjects

Among 742 patients screened between October 2015 and December 2016, 143 were excluded during screening, and 599 were randomized to receive therapy with AL or DHA-PQ (Figure 1). Twenty-one patients withdrew prior to completion of the study; 578 (96.5%) completed follow-up either through 42 days, if they did not develop parasitemia, or until detection of parasitemia (range, 14–42 days after initiation of therapy). Study populations assigned to the 2 treatment arms were similar at all 3 sites, but hemoglobin levels were significantly lower in the DHA-PQ arm at the Gulu site (Table 1).

Comparative Efficacies of Malaria Treatment Regimens

There were no early treatment failures. Compared with AL recipients, there was a lower cumulative risk of recurrent parasitemia among children who received DHA-PQ when assessed at 28 (32.0% vs 3.8%; $P < .001$) or 42 (47.0% vs 26.0%; $P < .001$) days (Table 2 and Figure 2). Findings were consistent across sites, with significantly fewer recurrent infections in the DHA-PQ arm at all 3 sites (Table 2). Genotyping to assign treatment outcomes was conclusive for 216 of 228 recurrent infections (94.7%). Corrected outcomes could not be determined for 12 recurrent infections (6 among AL recipients and 6 among DHA-PQ recipients). Eighteen recurrent infections (7.9%) were due to nonfalciparum parasitemia, which included *Plasmodium ovale* (n = 14), *Plasmodium malariae* (n = 3), and *Plasmodium vivax* (n = 1); these infections were censored at the time of parasitemia. The genotype-adjusted risks of recrudescent parasitemia were low for both AL and DHA-PQ recipients after 28 (1.7% and 0%, respectively; $P = .03$) and 42 (2.2% and 1.1%, respectively; $P = .25$) days.

Parasite clearance half-life could be determined for 587 participants (98.0%). The half-life could not be calculated for 12 individuals because of insufficient time points prior to clearance

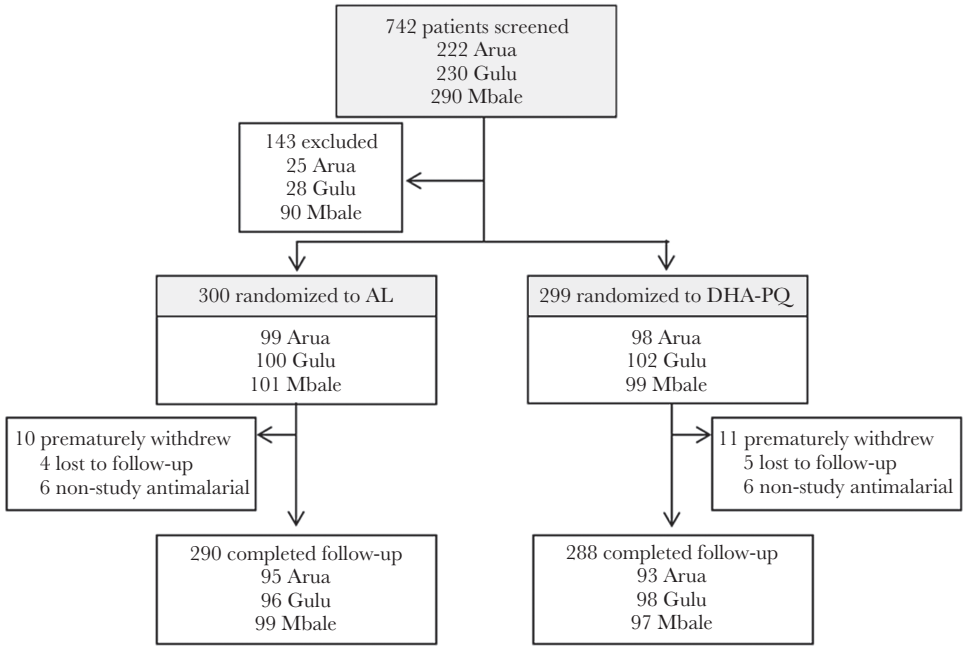

Figure 1. Trial profile. AL, artemether-lumefantrine; DHA-PQ, dihydroartemisinin-piperaquine.

Table 1. Baseline Characteristics of Study Participants

Characteristic, Location	Treatment Arm		*P*
	AL (n = 300)	DHA-PQ (n = 299)	
Female sex			
Arua	48.5	45.9	.72
Gulu	54.0	48.0	.40
Mbale	44.6	50.5	.40
Age, y, median (IQR)			
Arua	2.0 (1.3–3.6)	2.3 (1.3–3.5)	.63
Gulu	2.9 (1.4–3.9)	2.2 (1.4–3.3)	.27
Mbale	2.3 (1.5–3.5)	2.5 (1.5–3.8)	.51
Temperature, ◦C, mean ± SD			
Arua	37.9 ± 1.2	37.8 ± 1.2	.48
Gulu	38.1 ± 1.2	38.0 ± 1.2	.36
Mbale	38.1 ± 1.1	38.1 ± 1.2	.83
Parasite density, parasites/µL, geometric mean (range)			
Arua	41 074 (2020–210 360)	35 419 (2020–205 320)	.32
Gulu	62 125 (2380–193 360)	48 071 (2600–190 560)	.08
Mbale	31 368 (2160-198 200)	34 643 (2220–199 120)	.59
Gametocytes present, % of blood smears			
Arua	2.0	2.0	1.00
Gulu	2.0	6.9	.17
Mbale	16.8	10.2[a]	.22
Hemoglobin level, g/dL, mean ± SD			
Arua	10.2 ± 1.7	9.8 ± 1.8	.18
Gulu	10.3 ± 1.5	9.5 ± 1.6	.001
Mbale	10.0 ± 1.8	10.2 ± 1.6	.34

Data are percentage of participants, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; DHA-PQ, dihydroartemisinin-piperaquine; IQR, interquartile range

[a]Data were not available for 1 participant.

(n = 3), a parasitemia level >1000/µL at the last time point (n = 3), or inability to fit a linear segment to the data (n = 6). The median parasite clearance half-life was 2.7 hours for both AL and DHA-PQ recipients (P = .34), with a range of 1.0 to 9.8 hours for AL recipients and 1.1 to 8.3 hours for DHA-PQ recipients. Study site and enrollment parasite density were not associated with significant differences in parasite clearance half-life. The percentage of individuals with a parasite clearance half-life of >5 hours was similar for AL and DHA-PQ recipients (3.0% and 2.8%, respectively; P = .84). There were no significant differences in half-life when comparing sites (P = .78) or age (P = .64). Parasitemia on day 3 was very uncommon (1.0% in both treatment arms).

Adverse Events

The most common adverse events were cough, diarrhea, and abdominal pain (Table 3). None of the reported adverse events were attributed to the study drugs. Grade 3 or 4 adverse events occurred in 12 patients and included anemia (in 3), respiratory distress (in 4), bronchopneumonia (in 4), and vomiting/diarrhea (in 1), with 6 of these events each in the AL and DHA-PQ arms (P = 1.0).

Selection of *P. falciparum* Genetic Polymorphisms

We assessed *P. falciparum* genetic polymorphisms previously associated with antimalarial susceptibility in parasites isolated at enrollment and at the time of recurrence (Table 4). The prevalences of 3 mutations associated with resistance to and

Table 2. Treatment Outcomes

Outcome	All 3 Sites			Arua			Gulu			Mbale		
	AL (n = 300)	DHA-PQ (n = 299)	*P*	AL (n = 99)	DHA-PQ (n = 98)	*P*	AL (n = 100)	DHA-PQ (n = 102)	*P*	AL (n = 101)	DHA-PQ (n = 99)	*P*
Parasitemia												
Day 1	245 (81.7)	219 (73.2)	.01	79 (79.8)	59 (60.2)	.003	85 (85.0)	81 (79.4)	.30	81 (80.2)	79 (79.8)	.94
Day 2	34 (11.3)	22 (7.4)	.10	9 (9.1)	1 (1.0)	.02	12 (12.0)	11 (10.8)	.83	13 (12.9)	10 (10.1)	.66
Day 3	3 (1.0)	3 (1.0)	1.0	0	0	-	3 (3.0)	3 (2.9)	1.0	0	0	-
Parasite clearance half-life, h, median (IQR)[a]	2.7 (2.2–3.3)	2.7 (2.1–3.2)	.34	2.6 (2.1–3.2)	2.7 (2.2–3.1)	.79	2.6 (2.2–3.3)	2.6 (2.2–3.4)	.98	2.8 (2.4–3.4)	2.7 (2.2–3.1)	.12
Gametocytes present												
Day 1	20 (6.7)	18 (6.0)	.75	3 (3.0)	2 (2.0)	1.0	2 (2.0)	6 (5.8)	.28	15 (14.9)	10 (10.1)	.39
Day 2	13 (4.3)	14 (4.7)	.85	1 (1.0)	1 (1.0)	1.0	2 (2.0)	5 (4.9)	.45	10 (9.9)	8 (8.1)	.81
Day 3	6 (2.0)	8 (2.7)	.79	0	1 (1.0)	.50	1 (1.0)	6 (5.9)	.12	5 (5.0)	1 (1.0)	.21
Day 4–42	7 (2.3)	3 (1.0)	.34	2 (2.0)	0	.50	2 (2.0)	3 (3.0)	1.0	3 (3.0)	1 (1.0)	.25
Fever												
Day 1	231 (77.0)	208 (69.6)	.04	70 (70.7)	48 (49.0)	.002	90 (90.0)	85 (83.3)	.22	71 (70.3)	75 (75.8)	.43
Day 2	73 (24.3)	71 (23.8)	.92	11 (11.1)	5 (5.1)	.19	51 (51.0)	51 (50.0)	.89	11 (10.9)	15 (15.2)	.41
Day 3	18 (6.0)	31 (10.4)	.07	4 (4.0)	3 (3.1)	1.0	14 (14.1)	26 (25.5)	.05	0	2 (2.0)	.24
Hemoglobin level recovery, g/dL, mean ± SD[b]	0.80 ± 1.80	1.30 ± 1.69	.01	0.10 ± 1.33	1.3 ± 1.81	<.001	1.4 ± 1.32	1.4 ± 1.32	.94	1.1 ± 2.11	1.21 ± 1.88	.73
WHO treatment outcome, no. (%)												
No outcome	10 (3.3)	11 (3.7)		4 (4.0)	5 (5.1)		4 (4.0)	4 (3.9)		2 (2.0)	2 (2.0)	
Early treatment failure	0	0		0	0		0	0		0	0	
Late clinical failure	50 (16.7)	32 (10.7)		11 (11.1)	5 (5.1)		27 (27.0)	14 (13.7)		12 (11.9)	13 (13.1)	
Late parasitological failure	85 (28.3)	43 (14.4)		26 (26.3)	11 (11.2)		38 (38.0)	22 (21.6)		21 (20.8)	10 (10.1)	
Adequate response	155 (51.7)	213 (71.2)		58 (58.6)	77 (78.6)		31 (31.0)	62 (60.8)		66 (65.3)	74 (74.8)	
Cumulative risk of recurrent parasitemia, %[c]	47.0	26.0	<.001	38.9	17.0	<.001	68.6	37.1	<.001	33.6	23.6	.03
Cumulative risk of recrudescence, %	2.2	1.1	.25	0	1.1	.79	5.1	1.1	.11	2.3	1.2	.45

Data are no. (%) of participants, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; DHA-PQ, dihydroartemisinin-piperaquine; WHO, World Health Organization.

[a]Data are for 587 subjects.

[b]Data were available at enrollment and at day 42, among individuals without recurrence, for 124 subjects in the AL group and 187 subjects in the DHA-PQ group.

[c]Data are through day 42. Recurrences without genotyping data were censored at the time of parasitemia.

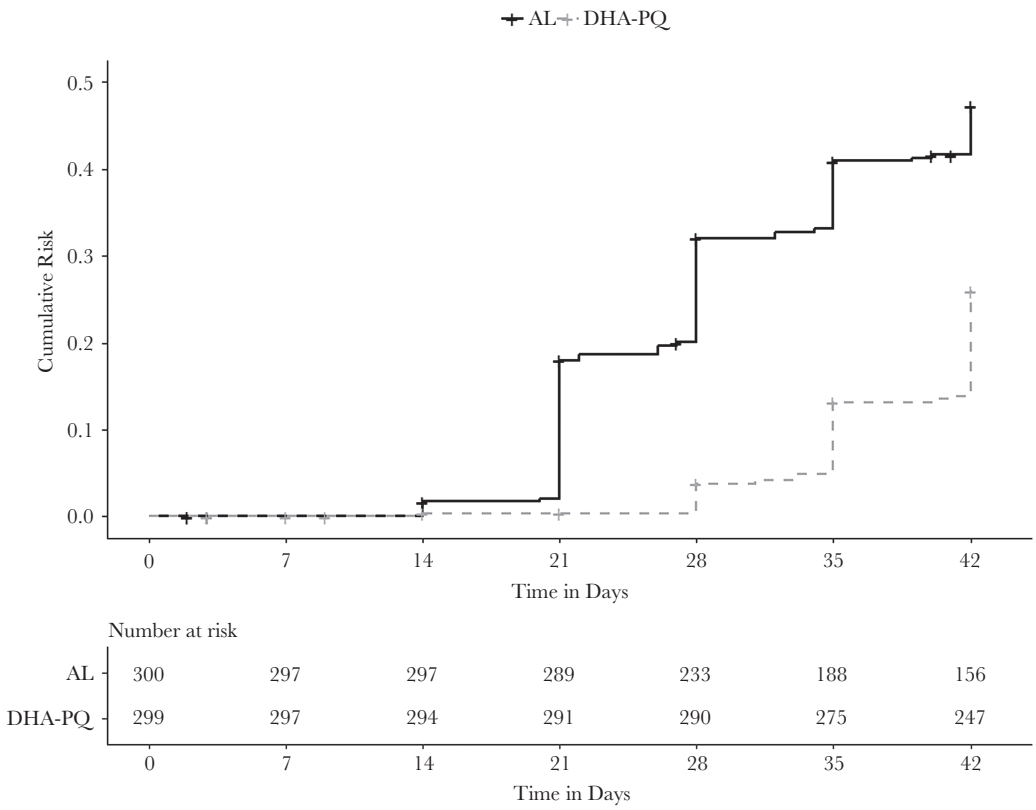

Figure 2. Cumulative risk of treatment failure unadjusted by genotype, by treatment arm. AL, artemether-lumefantrine; DHA-PQ, dihydroartemisinin-piperaquine.

selection by aminoquinolines, *pfcrt* 76T and *pfmdr1* 86Y and 1246Y, were all lower than those reported in prior surveys from Uganda, indicating continued decreases in the prevalences of these polymorphisms with replacement of chloroquine-based regimens with AL in Uganda [7–9]. In prior studies, treatment with ACTs was accompanied by selection of mutant (with AS/AQ and DHA-PQ) or wild-type (with AL) *pfcrt* and/or *pfmdr1* alleles [10–12]. In contrast, we identified no significant differences in the prevalence of the noted polymorphisms between parasites isolated from enrollment samples and those isolated at the time of recurrent malaria.

Table 3. Most-Common Adverse Events

	Treatment Arm, Proportion (%)		
Adverse Event	AL	DHA-PQ	*P*
Overall	264/300 (88)	275/299 (92)	.11
Cough	203/300 (68)	233/299 (78)	.01
Diarrhea	114/300 (38)	115/299 (38)	.91
Abdominal pain	45/125 (36)	41/124 (33)	.63
Anorexia	85/300 (28)	76/299 (25)	.42
Vomiting	61/300 (20)	56/243 (19)	.62
Headache	24/122 (20)	18/115 (16)	.42
Rash	42/300 (14)	56/299 (19)	.12
Weakness	33/300 (11)	42/299 (14)	.26
Pruritis	16/300 (5.3)	24/299 (8.0)	.19
Pallor	13/300 (4.3)	22/299 (7.4)	.12
Severe[a]	6/300 (2.0)	6/299 (2.0)	1.0

Abbreviations: AL, artemether-lumefantrine; DHA-PQ, dihydroartemisinin-piperaquine.
[a]Grade 3 or 4.

DISCUSSION

We compared the efficacies of AL and DHA-PQ for the treatment of uncomplicated malaria in children at 3 sites in Uganda. The risk of recrudescent malaria within 42 days after treatment was <3% for both arms. As expected, compared with AL, DHA-PQ was associated with a significantly lower risk of recurrent infection, attributable to extended posttreatment prophylaxis due to the long half-life of piperaquine. Neither regimen selected for changes in the prevalence of *P. falciparum pfcrt* and *pfmdr1* polymorphisms associated with drug resistance. Thus, the 2 study regimens both offered excellent treatment efficacy without apparent selection of parasites with decreased drug susceptibility, strongly suggesting that either regimen remains appropriate for the treatment of uncomplicated malaria in Uganda.

Our results, showing excellent treatment efficacies for both AL and DHA-PQ, were consistent with prior studies in Uganda [27, 28] and elsewhere in Africa [1, 29]. Our trial included frequent blood sampling after initiation of therapy, allowing determination of the parasite clearance half-life, an estimate of

Table 4. Prevalence of *pfmdr1* and *pfcrt* Single-Nucleotide Polymorphisms Associated With Decreased Antimalarial Susceptibility

Locus, Drug	Participants, No.	Pure Mutant Genotype, No. (%)	Mixed Mutant Genotype, No. (%)	Mutant or Mixed Genotype, %	*P*
pfmdr1 N86Y					
Before treatment	226	0	2 (0.8)	0.9	
AL	125	0	1 (0.8)	0.8	.93
DHA-PQ	65	0	2 (3.1)	3.1	.18
pfmdr1 Y184F					
Before treatment	226	59 (26)	101 (45)	70.8	
AL	124	40 (32)	50 (40)	72.6	.81
DHA-PQ	65	24 (40)	19 (29)	66.2	.47
pfmdr1 D1246Y					
Before treatment	226	3 (1.3)	15 (6.6)	8.0	
AL	121	1 (0.5)	8 (6.6)	7.4	.86
DHA-PQ	63	1 (1.6)	7 (11)	12.7	.25
pfcrt K76T					
Before treatment	226	9 (4.0)	22 (9.7)	13.7	
AL	124	3 (2.4)	13 (11)	12.9	.83
DHA-PQ	64	2 (3.1)	7 (11)	14.1	.94

Abbreviations: AL, artemether-lumefantrine; DHA-PQ, dihydroartemisinin-piperaquine.

the rate of response to therapy that is unaffected by the initial parasite density [25]. Both AL and DHA-PQ had low median clearance half-lives of <3 hours. In both treatment arms, only 3% of subjects had a parasite clearance half-life >5 hours, which has been associated with decreased susceptibility to artemisinins [30], and only 1% were parasitemic at day 3. These results suggest that clinically relevant resistance to AL and DHA-PQ continues to be absent in Uganda, consistent with recent reports based on ex vivo drug susceptibility [8, 31] and the prevalence of markers of resistance to artemisinins and piperaquine [7, 8].

As seen in prior studies, DHA-PQ provided a longer period of posttreatment prophylaxis after therapy than AL [1], leading to a significantly lower incidence of recurrent malaria within 42 days of therapy. The lower incidence of recurrent malaria after therapy and the simpler, once-daily dosing scheme for DHA-PQ makes it an attractive option for treatment of malaria in high-transmission settings such as Uganda [32]. However, an argument against routine treatment of uncomplicated malaria with DHA-PQ is that, unlike lumefantrine, to which resistance has not been identified, resistance to piperaquine has led to failure of treatment with DHA-PQ in Southeast Asia [33]. Piperaquine is a bis-quinoline related to chloroquine and amodiaquine, and detectable piperaquine concentrations may be present many weeks after treatment [34]. Treatment with DHA-PQ has been associated with a modest increase in the prevalence of mutant *pfcrt* and *pfmdr1* polymorphisms, but this has not been associated with a change in response to treatment [10, 12, 35]. In our study, there was a remarkably low pretreatment prevalence of the relevant *pfcrt* (76T) and *pfmdr1* (86Y and 1246Y) mutations as compared to prevalences in prior years, consistent with changes seen in Uganda in recent years [7–9], and treatment with DHA-PQ was not associated with selection of aminoquinoline resistance–mediating mutations. A different mechanism for resistance to piperaquine—an increased number of plasmepsin gene copies—was recently identified in Southeast Asia [16], but this polymorphism was uncommon and not associated with piperaquine susceptibility in a recent study in Uganda [8]. Taken together, our results offer reassurance that, with current background prevalences of parasites with *pfcrt* and *pfmdr1* mutations in Uganda, DHA-PQ may not readily select for resistance. However, resistance to multiple antimalarials has spread to Africa from other regions, and diligent surveillance for resistance to ACTs in Africa remains a high priority.

AL, the first-line therapy for uncomplicated malaria in Uganda and many other African countries, continues to show excellent efficacy. Remarkably, true resistance to lumefantrine has not been reported, although with an increased prevalence of wild-type sequences at relevant *pfcrt* and *pfmdr1* alleles, susceptibilities to lumefantrine have decreased slightly in Uganda [8]. Even with this decreased activity, the antimalarial efficacy of AL remained excellent. Importantly, as widespread AL use has shifted the prevalence of relevant genotypes toward wild type in Uganda, the previously inferior regimen AS/AQ [21] recently showed efficacy better than that of AL [13].

After exclusion of recurrent infections, DHA-PQ was associated with a 0.5-g/dL greater recovery in the hemoglobin level ($P = .01$) and a trend toward lower a prevalence of anemia (19% vs 25%; $P = .2$) on day 42, compared with AL. A prior study in Uganda also showed improved recovery in the hemoglobin level with DHA-PQ as compared to AL [28], and there was a trend toward improved recovery in the hemoglobin level for DHA-PQ as compared to other antimalarials in a meta-analysis considering 4 African trials [1]. Differences between the regimens might be due to more-rapid resolution of parasitemia after treatment with DHA-PQ (Table 2), but confirmation of this hypothesis would require additional data. With only modest differences in hemoglobin levels after therapy and limited available data to determine causation, we would not change treatment recommendations on the basis of these findings.

In summary, we found that AL and DHA-PQ were effective treatments for uncomplicated malaria among children in Uganda. DHA-PQ was associated with a significantly lower risk of recurrent malaria, and although posttreatment selection for genetic markers of chloroquine and amodiaquine resistance by DHA-PQ has been observed in the past, this study did not show an association. Thus, AL and DHA-PQ remain excellent treatment choices for uncomplicated malaria. However, with resistance emerging in other regions of the world, continued ACT treatment efficacy studies are a high priority.

Notes

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