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MAJOR ARTICLE

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(See the editorial commentary by Price on pages 1627–9.)

Background. Artemisinin-based combination therapy (ACT) reduces the potential for malaria transmission, compared with non-ACTs. It is unclear whether this effect differs between ACTs.

Methods. A total of 298 children (age, 6 months to 10 years) with uncomplicated falciparum malaria were randomized to artemether-lumefantrine (AL; n = 153) or dihydroartemisinin-piperaquine (DP; n = 145) in Mbita, a community in western Kenya. Gametocyte carriage was determined by molecular methods on days 0, 1, 2, 3, 7, 14, 28, and 42 after treatment initiation. The gametocyte infectiousness to mosquitoes was determined by mosquito-feeding assays on day 7 after beginning therapy.

Results. The cumulative risk of recurrent parasitemia on day 42 after initiation of treatment, unadjusted by polymerase chain reaction findings, was 20.7% (95% confidence interval [CI], 14.4–28.2) for AL, compared with 3.7% (95% CI, 1.2–8.5) for DP ($P < .001$). The mean duration of gametocyte carriage was 5.5 days (95% CI, 3.6–8.5) for AL and 15.3 days (95% CI, 9.7–24.2) for DP ($P = .001$). The proportion of mosquitoes that became infected after feeding on blood from AL-treated children was 1.88% (43 of 2293), compared with 3.50% (83 of 2371) for those that fed on blood from DP-treated children ($P = .06$); the oocyst burden among mosquitoes was lower among those that fed on blood from AL-treated children ($P = .005$)

Conclusions. While DP was associated with a longer prophylactic time after treatment, gametocyte carriage and malaria transmission to mosquitoes was lower after AL treatment.

Clinical Trials Registration: NCT00868465.

Keywords. malaria; falciparum; artemisinin; coartem; transmission; anopheles; mosquito; recrudescence; genotyping; gametocyte.

Artemisinin-based combination therapy (ACT) has been adopted as first-line treatment for uncomplicated malaria throughout Africa. Despite concerns about increasing resistance to artemisinins in Thailand and Cambodia [1, 2], ACTs continue to have excellent cure rates in Africa [3]. An important benefit of ACTs is their effect on gametocytes, which, when ingested by mosquitoes, trigger sexual reproduction of parasites. Gametocyte carriage and posttreatment malaria transmission is reduced after receipt of ACTs [4–6]. These transmission-reducing properties have been associated with sharp reductions in malaria transmission intensity and malaria incidence in several African settings following the

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introduction of ACTs [7–10]. While there is indisputable evidence that, compared with non-ACTs, ACT reduces the potential for malaria transmission [11], it is currently unclear whether this transmission-reducing effect differs among ACTs. Artemether-lumefantrine (AL) is the most widely used ACT in Africa. Dihydroartemisinin-piperaquine (DP) may be equally efficacious [3, 12] and has advantages of simpler dosing and a longer prophylactic period [3, 12].

There is conflicting evidence about the comparative effect of AL and DP on posttreatment gametocyte carriage. Some studies indicate a longer duration of microscopically detected gametocyte carriage after AL treatment [12–14], whereas others indicate a similar [15] or shorter duration [3, 16, 17]. A large multicenter ACT trial indicated that definitive conclusions about the malaria transmission potential after different ACTs are currently unavailable and cannot be based on microscopy alone [3] since microscopy only detects a fraction of all gametocytes [17]. Individuals with gametocyte densities below the microscopic threshold for detection have repeatedly been shown to be infectious to mosquitoes [18–20], and a minimum gametocyte density that is required for successful mosquito infection has not been established. Importantly, microscopy and other currently available gametocyte detection tools do not allow inferences on the infectiousness of gametocytes to mosquitoes to be made. The infectiousness of gametocytes that are observed after initiation of treatment may vary between treatment regimens: most antimalarials clear immature gametocytes but leave highly infectious mature gametocytes unaffected [21], whereas others appear to induce the production or release of less infectious gametocytes [6, 22, 23]. Mosquito-feeding assays are the only currently available tools to provide direct evidence of the infectiousness of gametocytes.

We used a highly sensitive molecular assay to determine the duration of gametocyte carriage among children who received AL or DP, and, to our knowledge, we are the first to have directly determined gametocyte transmission to mosquitoes that fed on posttreatment blood samples.

METHODS

Study Design and Participants

This study was conducted in Mbita, a community in western Kenya, from April to June 2009. The study area is characterized by moderate transmission intensity [4]. Previous trials with AL and DP in 2004 and 2007 found an adequate clinical response over 28 days in 82%–100% of treated children, with all patients exhibiting microscopy-confirmed clearance of parasitemia by day 2 after initiation of treatment [4, 14]. Children from 6 months 10 years old were included when they had either a tympanic temperature of ≥37.5°C or a history of fever in the last 24 hours and microscopically confirmed *Plasmodium falciparum* infection with an asexual parasite density of 1000–200 000 parasites/μL. Exclusion criteria were a hemoglobin level of <5 g/dL, the presence of other disease that causes febrile conditions, the presence of any other *Plasmodium* species, a history of adverse events against either of the study drugs, or signs of severe malaria. The protocol received ethical approval from the Ethical Review Committee of the Kenya Medical Research Institute and the ethics committee of the London School of Hygiene and Tropical Medicine. Written informed consent was obtained from a parent or guardian of the participating children.

Interventions and Randomization

Children were weighed and randomly allocated to receive (1) AL (Coartem; Novartis Pharma) administered as half a tablet (20 mg of artemether and 120 mg of lumefantrine) per 5 kg of body weight in a 6-dose regimen (at enrollment and 8, 20, 32, 44, and 56 hours [±90 minutes] after initiation of treatment) or (2) DP (Duocotexin, Holley Pharm, 40 mg dihydroartemisinin/320 mg piperaquine tablets) administered as a targeted total dose of 6.4 and 51.2 mg/kg of dihydroartemisinin and piperaquine, respectively, given in 3 equally divided daily doses to the nearest half tablet. The quality of the drugs used in this trial was confirmed by the methods described by Kaur et al [24]. All treatment doses were given under direct supervision with local fatty food to facilitate absorption. A randomization list was generated for different age strata (<2 years, 2–5 years, and 5–10 years), using MS Excel, targeting allocation to each of the study arms at a ratio of 1:1. Except for those involved in administering medication, all staff members engaged in the trial were blinded to the treatment arm to which each child was assigned.

Procedures

Children were examined at the study clinic on days 1, 2, 3, 7, 14, 28, and 42 after enrollment and on any other day that the child became ill. If children were parasitemic at any time point after day 3, they received rescue treatment with mefloquine and were excluded from further follow-up. A single finger-stick specimen was collected on all follow-up days after enrollment and was used for preparation of a microscopic slide (on all days except day 1), a 50-μL microtainer blood sample, and a filter paper blood spot (903 and 3MM Whatman, Maidstone, UK). All blood smears were Giemsa stained, and 100 microscopic fields were read by 2 microscopists for asexual parasites, with a third microscopist used if results differed by >25%. At enrollment and on day 7, the day of membrane feeding, slides were re-read for gametocytes by 2 independent microscopists. Gametocyte detection was done for a random selection of individuals on all days of follow-up by quantitative nucleic acid sequence–based amplification (QT-NASBA) as described elsewhere, with an approximate detection limit of 0.1 gametocytes/μL [25]. MSP-1 and MSP-2 genotyping was performed using primers described by Snounou et al [26] after DNA extraction by the Chelex method [27] or the QiaAmp DNA Micro kit (Qiagen, UK) to

distinguish between recrudescence and reinfections on paired filter paper samples from enrollment and the follow-up day on which parasites were detected by microscopy [28]. These 2 markers were previously shown to be discriminative in the study region [4, 29].

On day 7 after initiation of treatment, all children aged ≥2 years were invited for membrane-feeding assays. Additional written informed consent was obtained from a parent or guardian of children participating in membrane-feeding assays. Children were enrolled in membrane-feeding assays in the order in which they appeared at the clinic; the maximum number of experiments that was conducted per day depended on mosquito husbandry. A 3-mL venous blood sample was obtained; if venipuncture failed twice, the child was not enrolled in the membrane-feeding experiment. Blood samples were fed to approximately 100 locally reared 4–5-day-old female *Anopheles gambiae sensu stricto* mosquitoes via an artificial membrane-feeder system [4]. One week later, up to 30 surviving mosquitoes per experiment were examined for oocysts by 2 independent microscopists. A third microscopist was consulted if the 2 microscopists disagreed, and the majority result was recorded.

Sample Size Calculations

We calculated a sample size that was sufficient to test the hypothesis that the risk of recurrent parasitemia after 42 days would differ between AL-treated children and DP-treated children. A total of 150 patients (allowing for 10% loss to follow-up) needed to be enrolled in each treatment arm to detect a difference of 20% in the risk of recurrent parasitemia, assuming a 42-day risk of recurrent parasitemia (unadjusted by genotyping) of 50% after AL treatment [30], with a 2-sided type I error of 0.05 and a power of 90%. A random selection was made for QT-NASBA analysis, using computer-randomized tables. The number of individuals included in QT-NASBA analysis was based on an estimated duration of gametocyte carriage (±SD) of 13.4 ± 7.5 days [31] after AL treatment, with a 65% longer duration of carriage after DP treatment [3]. Inclusion of 47 children per treatment arm, each contributing 1 estimate of the duration of gametocyte carriage, would allow us to detect this difference with a 2-sided type I error of 0.05 and a power of 95%. The number of children included in membrane-feeding assays was not based on sample size calculations but on the maximum that was logistically feasible on the basis of mosquito husbandry. Previous studies have included 10–61 experiments per treatment arm [4, 5].

Data Analysis

The primary outcome was the parasitological efficacy of AL and DP. The time to treatment failure, defined as the time to the appearance of asexual parasites during follow-up, without adjustment for polymerase chain reaction (PCR) findings, was compared between treatment arms by Cox proportional hazard models. The number of PCR-confirmed recrudescence was compared between treatment arms by the Fisher exact test and logistic regression models; for this purpose, only infections that were classified as recrudescence after PCR adjustment by MSP-1 and MSP-2 genotyping findings were considered treatment failures.

Secondary outcomes were (submicroscopic) gametocyte carriage and malaria transmission to mosquitoes. The mean duration of gametocyte carriage after treatment for individuals with an adequate clinical response was estimated using a previously published mathematical model for repeated QT-NASBA measurements [31]. The main advantage of this model is that it allows for the release of gametocytes from sequestration. The disappearance of gametocytes during follow-up for individuals who carried gametocytes at enrollment, as detected by QT-NASBA, was determined using a Kaplan-Meier estimator; the log-rank test was used to compare curves for AL and DP. The proportion of gametocytemic individuals and the proportion of infectious individuals (ie, subjects who infected at least 1 mosquito) were compared between treatment arms by the χ^2 test and logistic regression models. The proportion of infected mosquitoes, the oocyst burden in mosquitoes, and the number of gametocyte-positive days were compared between groups by negative binomial or logistic regression models, using generalized estimating equations to adjust for clustering between observations from the same individual.

Because the dose of DP received was previously associated with the risk of recrudescence or reinfection [32, 33], we calculated the total actual doses of lumefantrine and piperaquine received over the 3 treatment days and related this to treatment outcome.

RESULTS

Of the 2073 screened children, 298 met the enrollment criteria and were randomly assigned to receive treatment with AL or DP (Figure 1). Geometric mean asexual parasite density at enrollment was 15 360 parasites/μL (95% confidence interval [CI], 13 432–17 564 parasites/μL) and did not differ between treatment arms ($P = .28$; Table 1). Enrollment gametocyte prevalence was 9.7% (26 of 267) by microscopy and 71.3% (67 of 94) by QT-NASBA and did not differ between treatment arms ($P \geq .49$; Table 1). The mean total dose of lumefantrine in the AL arm (±SD) was 65.5±8.4 mg/kg body weight (range, 51.4–102.9 mg/kg body weight); the mean total dose of piperaquine in the DP arm (±SD) was 60.2±10.7 mg/kg body weight (range, 48.0–87.3 mg/kg body weight).

Primary Analysis: Efficacy of AL and DP

By day 42 after initiation of treatment, 20.7% of the children (30 of 145) in the AL arm were parasite positive by microscopy, compared with 3.7% (5 of 134) in the DP arm (hazard ratio, 0.17; 95% CI, .07–.44; $P < .001$; Table 2). This failure rate was

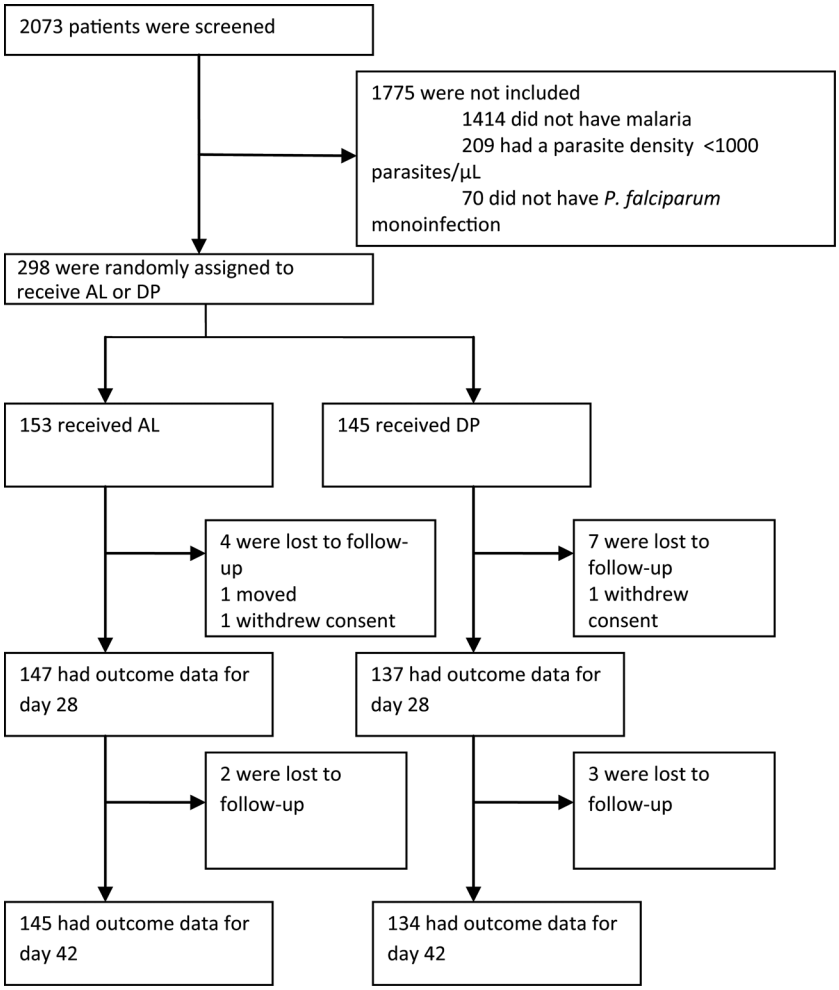

Figure 1. Trial profile. Abbreviation: *P. falciparum, Plasmodium falciparum*

not statistically significantly associated with enrollment parasite density ($P = .38$), age ($P = .94$), total dose of lumefantrine ($P = .31$), or total dose of piperaquine ($P = .64$). MSP-1 and MSP-2 genotyping was successful for 30 of 35 recurrent infections; 3 samples failed to amplify on the day of failure or the preceding visit, and 2 infections had missing samples. Only 4 of these 30 samples, all from the AL arm ($P = .053$), were classified as recrudescence, and the remainder were classified as new infections.

By microscopy, all children cleared their asexual parasites by day 7 after treatment. On day 2 after treatment, before the fifth dose of AL or the third dose of DP was administered, 3.3% of children (5 of 150) in the AL arm and 7.3% (10 of 138) in the DP arm had residual asexual parasitemia detected by microscopy ($P = .14$). On day 3 after initiation of treatment, 1 child in the DP arm had residual asexual parasitemia of 150 parasites/μL detected by microscopy, down from an initial asexual parasite density of 3500 parasites/μL.

Secondary Analysis: Posttreatment Malaria Transmission Potential

QT-NASBA–determined gametocyte prevalence declined during follow-up (Figure 2). The gametocyte prevalence increased on days 28 and 42 of follow-up and was strongly associated with the concurrent presence of asexual parasites on these days. On day 42 after initiation of treatment, the QT-NASBA–determined gametocyte prevalence was 45.5% (5 of 11) for children who had asexual parasites detected by microscopy on that day, compared with 5.4% (2 of 37) for parasite-free children ($P = .001$). The number of gametocyte-positive days was lower for AL-treated children (32.0% [110 of 344]) than for DP-treated children (42.9% [127 of 296]; $P = .008$). The mean duration of gametocyte carriage for children who remained free of asexual parasites during follow-up was 5.5 days (95% CI, 3.6–8.5) for the AL group and 15.3 days (95% CI, 9.7–24.2) for the DP group ($P = .001$). Some individuals harbored gametocytes considerably longer (Figure 3). When analyses were restricted to individuals

Table 1. Characteristics of the Study Participants at Enrollment, by Study Arm

Variable	AL	DP
Children, no.	153	145
Male sex	51.0 (78/153)	51.0 (74/145)
Age, y, median (IQR)	5 (3–8)	5 (3–7)
Temperature ≥37.5°C	43.1 (66/153)	41.4 (60/145)
Hemoglobin level, mmol/dL, mean (95% CI)	6.58 (6.39–6.78)	6.40 (6.20–6.61)
Asexual parasite density, parasites/μL, geometric mean (95% CI)	15 840 (13 213–18 990)	15 160 (12 415–18 512)
Microscopy finding		
Gametocyte prevalence	8.8 (12/137)	10.8 (14/130)
Gametocyte density,[a] gametocytes/μL, geometric mean (95% CI)	64.0 (33.7–121.5)	64.7 (37.2–112.4)
Pfs25 QT-NASBA finding		
Gametocyte prevalence	68.1 (32/47)	74.5 (35/47)

Data are % (proportion) of participants, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DP, dihydroartemisinin-piperaquine; IQR, interquartile range; QT-NASBA, quantitative nucleic acid sequence–based amplification.

[a] Data are for gametocyte carriers only.

who were gametocyte positive by QT-NASBA at enrollment, the time to disappearance of gametocytes was significantly shorter for the AL group, compared with the DP group (hazard ratio, 2.35; 95% CI, 1.19–4.66; $P = .01$; Figure 3).

One hundred and sixty-two children agreed to donate blood for membrane-feeding experiments. Venipuncture failed for 5 children; for the other 157 experiments, a minimum of 17 mosquitoes were examined on day 7 after feeding (median, 30

Table 2. Treatment Outcomes on Days 28 and 42 After Initiation of Treatment, by Study Arm

Outcome, by Day	AL	DP	*P*
Day 28	147	137	
Observations, no.			
Treatment outcome			
Adequate clinical response	93.2 (137/147)	100.0 (0/137)	.002
Early treatment failure	0.0 (0/147)	0.0 (0/147)	
Late treatment failure			
Overall[a]	6.8 (10/147)	0.0 (0/137)	
Due to recrudescence[b]	1.4 (2/147)	0.0 (0/137)	
Due to new infection[b]	5.4 (8/147)	0.0 (0/137)	
Indeterminate cause	0.0 (0/147)	0.0 (0/137)	
Day 42			
Observations, no.	145	134	
Treatment outcome day 42, %			
Adequate clinical response	79.3 (115/145)	96.3 (129/134)	<.001
Early treatment failure	0.0 (0/145)	0.0 (0/134)	
Late treatment failure			
Overall[a]	20.7 (30/145)	3.7 (5/134)	
Due to recrudescence[b]	2.8 (4/145)	0.0 (0/134)	
Due to new infection[b]	15.9 (23/145)	2.2 (3/134)	
Indeterminate cause	2.1 (3/145)	1.5 (2/134)	

Data are % (proportion) of observations, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine; PCR, polymerase chain reaction.

[a] Unadjusted by PCR findings.

[b] Adjusted by PCR-based MSP-1 and MSP-2 genotyping findings for samples collected at enrollment and on the day of failure.

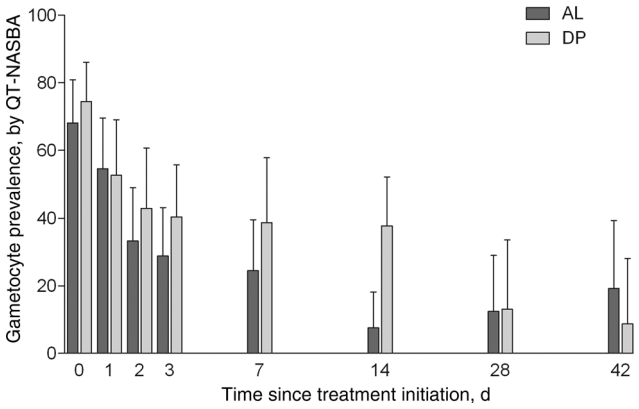

Figure 2. Gametocyte prevalence by quantitative nucleic acid sequence–based amplification (QT-NASBA) after initiation of treatment with artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DP). RNA samples were processed for gametocyte detection by Pfs25 QT-NASBA at enrollment and on days 1, 2, 3, 7, 14, 28, and 42 after initiation of treatment with AL (n = 47) or DP (n = 47). Gametocyte prevalence is given for the different treatment arms; error bars indicate the upper limit of the 95% confidence interval.

mosquitoes; interquartile range, 30–30 mosquitoes). For 151 of these experiments, slide results were available; 80.0% of children (12 of 15) who had gametocytes detected by microscopy in the feed sample infected at least 1 mosquito, compared with 29.4% (40 of 136) with slides that were negative for gametocytes ($P < .001$). Gametocyte carriage detected by QT-NASBA was associated with the likelihood of infecting at least 1 mosquito. Of children who were gametocyte positive by QT-NASBA, 66.7% (18 of 26) infected at least 1 mosquito, compared with 23.7% of children (9 of 38) who were gametocyte negative by QT-NASBA ($P < .001$). Infectiousness to mosquitoes was not significantly associated with fever at enrollment ($P = .44$) or fever on the day of membrane feeding ($P = .73$).

The proportion of individuals that infected at least 1 mosquito was not significantly different between treatment arms ($P = .40$; Table 3). However, the proportion of infected mosquitoes and oocyst intensity were different between treatment arms. In the AL arm, 1.9% of mosquitoes (44 of 2293) became infected with 1–2 oocysts per mosquito midgut. In the DP arm, 3.5% of mosquitoes (84 of 2371) became infected with 1–14 oocysts. Treatment with DP was associated with a borderline significantly higher proportion of infected mosquitoes (odds ratio, 1.96; 95% CI, .96–3.97; $P = .06$) and a significantly higher oocyst burden in infected mosquitoes (incidence rate ratio, 2.71; 95% CI, 1.34–5.47; $P = .005$), after adjustment for correlation between observations from the same individual.

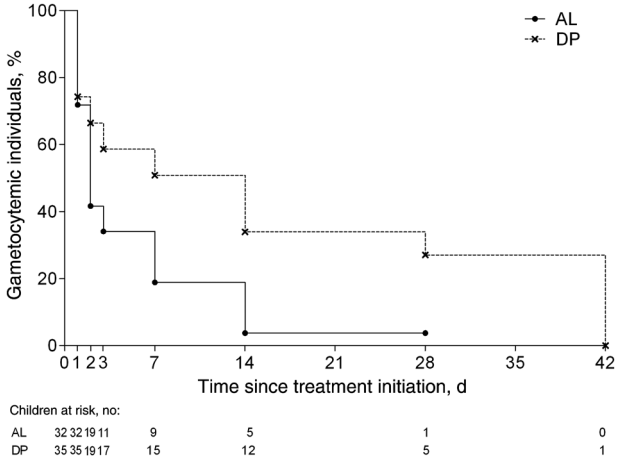

Figure 3. Kaplan-Meier plot of the time to disappearance of gametocytes among individuals who were gametocyte positive before treatment with artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DP). The time to disappearance of gametocytes was determined for individuals who were gametocyte positive by Pfs25 quantitative nucleic acid sequence–based amplification prior to AL treatment (solid line; n = 32) or DP treatment (dashed line; n = 35). $P = .014$, by the log-rank test.

DISCUSSION

In this study, we present the first direct comparison of the malaria transmission–reducing effects of 2 leading ACTs. Compared with DP, treatment with AL was associated with a 3-fold shorter duration of gametocyte carriage after initiation of treatment and a significantly lower infectiousness to mosquitoes on day 7 after initiation of treatment.

In line with a recent multicenter study on the efficacy of ACTs in Africa, we found high efficacies of AL and DP [3]. While 35 individuals experienced parasitemia after initial parasite clearance by treatment, MSP-1 and MSP-2 genotyping confirmed only 4 cases of recrudescence, all of which occurred in the AL arm. DP thereby had a significantly higher efficacy than AL. In addition to a better efficacy in preventing recrudescence, our findings also indicated a lower rate of reinfections in the DP arm [3, 12, 13, 15]. This finding is in line with all published literature on the prophylactic period after ACTs and is plausibly a result of the longer elimination half-life of piperaquine (23–28 days), compared with that for lumefantrine (3.2 days) [34, 35]. The longer prophylactic period following treatment is an important advantage for the individual patient in areas of moderate-to-intense malaria transmission where reinfection is likely [36], but it may result in a longer period with subtherapeutic drug levels, during which selection for resistant parasites may occur [37]. In settings of low endemicity, the individual benefit of a longer prophylactic period is relatively small. Drug resistance is more likely to arise in areas of low endemicity [38]; there may therefore be disadvantages of using drugs with a long elimination half-life in these settings. The gametocytocidal properties of ACTs are key in determining their impact on community-wide malaria transmission in settings where malaria

Table 3. Gametocyte Infectiousness Among Mosquitoes, by Study Arm

Variable	AL	DP	*P*
Individuals participating in membrane-feeding assays, no.	77	80	
Microscopy finding on feeding day			
Gametocyte prevalence	4.2 (3/72)	15.2 (12/79)	.02
Gametocyte density, gametocytes/μL, geometric mean (95% CI)[a]	39.5 (18.2–85.4)	63.8 (38.5–105.8)	.29
Pfs25 QT-NASBA finding on feeding day			
Gametocyte prevalence	21.7 (5/23)	39.1 (9/23)	.20
Individuals infecting ≥1 mosquito	31.1 (24/77)	36.3 (29/80)	.40
Infected mosquitoes, % (proportion)	1.9 (44/2293)	3.5 (84/2371)	.06[b]
Oocysts in infected mosquitoes, no., mean (range)	1.3 (1–2)	1.9 (1–14)	.005[b,c]

Data are % (proportion) of participants, unless otherwise indicated.

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DP, dihydroartemisinin-piperaquine; QT-NASBA, quantitative nucleic acid sequence–based amplification.

[a]Data are for gametocyte carriers only.

[b]Adjusted for correlations between observations from the same individual.

[c]Determined using a negative binomial regression model that incorporated both prevalence and intensity of infection among mosquitoes.

endemicity is low [39]. A long-acting drug with a strong gametocytocidal activity may have the largest impact across all levels of transmission intensity. DP has been suggested to fulfill this role as an ACT with a long-acting partner drug [39]. However, our study shows that the gametocytocidal effect of DP immediately after treatment was smaller than that of AL.

Gametocytes undergo complex development that is characterized by 5 morphologically distinct stages. The earliest developmental stages of gametocytes (stage I and II) are susceptible to most antimalarial drugs, including lumefantrine and piperaquine [40]. Later-stage gametocytes are unaffected by piperaquine and lumefantrine [40], although a recent study suggested an effect of lumefantrine on mature gametocytes [41]. The active metabolite of artemisinins, dihydroartemisinin, is highly active against stage I–III gametocytes and has incomplete activity against stage IV and V gametocytes [40, 42]. The artemisinin component is therefore likely to be most important in determining differences in transmission potential after AL and DP therapy. A recent multicenter trial comparing 4 different ACTs observed a significantly higher prevalence of microscopically detectable gametocytes after DP therapy but acknowledged that microscopy only detects a fraction of all gametocytes and does not allow definitive conclusions about malaria transmission potential [3]. We used a highly sensitive molecular gametocyte detection tool that, in line with previous studies, resulted in a 7-fold higher estimation of gametocyte prevalence at enrollment [17]. We observed that mature gametocytes can persist for several weeks after initiation of ACT treatment [31] and that the duration of gametocyte carriage was approximately 3-fold shorter for the AL arm, compared with the DP arm. Repeated assessments of gametocyte carriage by use of sensitive assays allow incorporation of a longitudinal element in the estimation of malaria transmission potential but do not provide conclusive evidence about the infectiousness of gametocytes. We therefore directly determined posttreatment infectiousness 1 week after the initiation of treatment in 157 of 298 children, an unsurpassed high proportion of trial participants. We previously showed that a large proportion of children are capable of infecting mosquitoes after ACT [4], owing the longevity of gametocytes [31] and the high efficiency of malaria transmission at low gametocyte densities [20]. As a consequence, the proportion of individuals capable of infecting at least 1 mosquito may be similar between treatment arms [4], whereas the proportion of mosquitoes that become infected after feeding, which is the transmission outcome with the largest public health importance, differs as a consequence of differences in gametocyte density and infectiousness. In this study, the proportion of mosquitoes that became infected after feeding on the blood of a treated individual was approximately 2-fold lower after AL treatment, compared with DP treatment. Ideally, we would have conducted longitudinal assessments of infectiousness after AL and DP therapy. This would have allowed us to determine the overall impact of these ACTs with different gametocytocidal and prophylactic properties on the malaria transmission potential in our study setting, where reinfection is frequent. The fact that many children with asexual parasites during follow-up concurrently harbored gametocytes indicates that rates of drug failure, be it due to recrudescence or reinfection, have direct implications for transmissibility. In *Plasmodium vivax*, this association is more readily appreciated because gametocytes develop early in infections [17], and a lower efficacy of AL as compared to DP results in an immediate increased *P. vivax* transmission potential after AL [43].

With the currently available data, we can speculate about the plausible impact of AL and DP on *P. falciparum* malaria transmission in different settings. Because of the more pronounced effect of AL on malaria transmission shortly after treatment, our findings suggest that AL may be the most appropriate first-line choice for reducing community-wide transmission of *P. falciparum* in settings of low endemicity. DP may be an appropriate choice to prevent reinfections in areas of higher endemicity.

Notes

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References

1. Phyo AP, Nkhoma S, Stepniewska K, et al. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet **2012**; 379:1960–6.
2. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med **2009**; 361:455–67.
3. 4ABC Study Group. A head-to-head comparison of four artemisinin-based combinations for treating uncomplicated malaria in African children: a randomized trial. PLoS Med **2011**; 8:e1001119.
4. Bousema JT, Schneider P, Gouagna LC, et al. Moderate effect of artemisinin-based combination therapy on transmission of *Plasmodium falciparum*. J Infect Dis **2006**; 193:1151–9.
5. Sutherland CJ, Ord R, Dunyo S, et al. Reduction of malaria transmission to anopheles mosquitoes with a six-dose regimen of co-artemether. PLoS Med **2005**; 2:e92.
6. Targett G, Drakeley C, Jawara M, et al. Artesunate reduces but does not prevent posttreatment transmission of *Plasmodium falciparum* to *Anopheles gambiae*. J Infect Dis **2001**; 183:1254–9.
7. Barnes KI, Chanda P, Ab Barnabas G. Impact of the large-scale deployment of artemether/lumefantrine on the malaria disease burden in Africa: case studies of South Africa, Zambia and Ethiopia. Malar J **2009**; 8(Suppl 1):S8.
8. Barnes KI, Durrheim DN, Little F, et al. Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. PLoS Med **2005**; 2:e330.
9. Bhattarai A, Ali AS, Kachur SP, et al. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS Med **2007**; 4:e309.
10. Price RN, Nosten F, Luxemburger C, et al. Effects of artemisinin derivatives on malaria transmissibility. Lancet **1996**; 347:1654–8.
11. Okell LC, Drakeley CJ, Ghani AC, Bousema T, Sutherland CJ. Reduction of transmission from malaria patients by artemisinin combination therapies: a pooled analysis of six randomized trials. Malar J **2008**; 7:125.
12. Bassat Q, Mulenga M, Tinto H, et al. Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial. PLoS One **2009**; 4:e7871.
13. Yeka A, Dorsey G, Kamya MR, et al. Artemether-lumefantrine versus dihydroartemisinin-piperaquine for treating uncomplicated malaria: a randomized trial to guide policy in Uganda. PLoS One **2008**; 3:e2390.
14. Mens PF, Sawa P, van Amsterdam SM, et al. A randomized trial to monitor the efficacy and effectiveness by QT-NASBA of artemether-lumefantrine versus dihydroartemisinin-piperaquine for treatment and transmission control of uncomplicated *Plasmodium falciparum* malaria in western Kenya. Malar J **2008**; 7:237.
15. Arinaitwe E, Sandison TG, Wanzira H, et al. Artemether-lumefantrine versus dihydroartemisinin-piperaquine for falciparum malaria: a longitudinal, randomized trial in young Ugandan children. Clin Infect Dis **2009**; 49:1629–37.
16. Smithuis F, Kyaw MK, Phe O, et al. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. Lancet Infect Dis **2010**; 10:673–81.
17. Bousema T, Drakeley C. Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clin Microbiol Rev **2011**; 24:377–410.
18. Boudin C, Olivier M, Molez JF, Chiron JP, Ambroise-Thomas P. High human malarial infectivity to laboratory-bred *Anopheles gambiae* in a village in Burkina Faso. Am J Trop Med Hyg **1993**; 48:700–6.
19. Ouedraogo AL, Bousema T, Schneider P, et al. Substantial contribution of submicroscopical *Plasmodium falciparum* gametocyte carriage to the infectious reservoir in an area of seasonal transmission. PLoS One **2009**; 4:e8410.
20. Schneider P, Bousema JT, Gouagna LC, et al. Submicroscopic *Plasmodium falciparum* gametocyte densities frequently result in mosquito infection. Am J Trop Med Hyg **2007**; 76:470–4.
21. Fofana B, Djimde AA, Sagara I, et al. Impact of artemisinin-based combination therapy on malaria transmission in Mali [abstract MIM16762172]. In: Program and abstracts of the 5th MIM Pan-African Malaria Conference, Nairobi, Kenya, 2–6 November **2009**: 5.
22. Hallett RL, Dunyo S, Ord R, et al. Chloroquine/sulphadoxine-pyrimethamine for Gambian children with malaria: transmission to mosquitoes of multidrug-resistant *Plasmodium falciparum*. PLoS Clin Trials **2006**; 1:e15.
23. Beavogui AH, Djimde AA, Gregson A, et al. Low infectivity of *Plasmodium falciparum* gametocytes to *Anopheles gambiae* following treatment with sulfadoxine-pyrimethamine in Mali. Int J Parasitol **2010**; 40:1213–20.
24. Kaur H, Goodman C, Thompson E, et al. A nationwide survey of the quality of antimalarials in retail outlets in Tanzania. PLoS One **2008**; 3: e3403.
25. Schneider P, Schoone G, Schallig H, et al. Quantification of *Plasmodium falciparum* gametocytes in differential stages of development by quantitative nucleic acid sequence-based amplification. Mol Biochem Parasitol **2004**; 137:35–41.
26. Snounou G, Zhu X, Siripoon N, et al. Biased distribution of msp1 and msp2 allelic variants in *Plasmodium falciparum* populations in Thailand. Trans R Soc Trop Med Hyg **1999**; 93:369–74.
27. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE. Pyrimethamine and proguanil resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. Am J Trop Med Hyg **1995**; 52: 565–8.

28. World Health Organization (WHO). Genotyping to identify parasite populations. Geneva: WHO, **2008**.
29. Waitumbi JN, Anyona SB, Hunja CW, et al. Impact of RTS,S/AS02(A) and RTS,S/AS01(B) on genotypes of *P. falciparum* in adults participating in a malaria vaccine clinical trial. PLoS One **2009**; 4:e7849.
30. Bukirwa H, Yeka A, Kamya MR, et al. Artemisinin combination therapies for treatment of uncomplicated malaria in Uganda. PLoS Clin Trials **2006**; 1:e7.
31. Bousema T, Okell L, Shekalaghe S, et al. Revisiting the circulation time of *Plasmodium falciparum* gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. Malar J **2010**; 9:136.
32. Price RN, Hasugian AR, Ratcliff A, et al. Clinical and pharmacological determinants of the therapeutic response to dihydroartemisinin-piperaquine for drug-resistant malaria. Antimicrob Agents Chemother **2007**; 51:4090–7.
33. Ashley EA, Krudsood S, Phaiphun L, et al. Randomized, controlled dose-optimization studies of dihydroartemisinin-piperaquine for the treatment of uncomplicated multidrug-resistant falciparum malaria in Thailand. J Infect Dis **2004**; 190:1773–82.
34. Hung TY, Davis TM, Ilett KF, et al. Population pharmacokinetics of piperaquine in adults and children with uncomplicated falciparum or vivax malaria. Br J Clin Pharmacol **2004**; 57:253–62.
35. Ezzet F, van Vugt M, Nosten F, Looareesuwan S, White NJ. Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria. Antimicrob Agents Chemother **2000**; 44:697–704.
36. Price RN, Douglas NM. Artemisinin combination therapy for malaria: beyond good efficacy. Clin Infect Dis **2009**; 49:1638–40.
37. Stepniewska K, White NJ. Pharmacokinetic determinants of the window of selection for antimalarial drug resistance. Antimicrob Agents Chemother **2008**; 52:1589–96.
38. Pongtavornpinyo W, Yeung S, Hastings IM, Dondorp AM, Day NP, White NJ. Spread of anti-malarial drug resistance: mathematical model with implications for ACT drug policies. Malar J **2008**; 7:229.
39. Okell LC, Drakeley CJ, Bousema T, Whitty CJ, Ghani AC. Modelling the impact of artemisinin combination therapy and long-acting treatments on malaria transmission intensity. PLoS Med **2008**; 5:e226; discussion e226.
40. Adjalley SH, Johnston GL, Li T, et al. Quantitative assessment of *Plasmodium falciparum* sexual development reveals potent transmission-blocking activity by methylene blue. Proc Natl Acad Sci U S A **2011**; 108:E1214–23.
41. van Pelt-Koops JC, Pett HE, Graumans W, et al. The spiroindolone drug candidate NITD609 potently inhibits gametocytogenesis and blocks *Plasmodium falciparum* transmission to *Anopheles* mosquito vector. Antimicrob Agents Chemother **2012**; 56:3544–8.
42. White NJ. Qinghaosu (artemisinin): the price of success. Science **2008**; 320:330–4.
43. Ratcliff A, Siswantoro H, Kenangalem E, et al. Two fixed-dose artemisinin combinations for drug-resistant falciparum and vivax malaria in Papua, Indonesia: an open-label randomised comparison. Lancet **2007**; 369:757–65.