Articles

◑ Amodiaquine-artesunate versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria in African children: a randomised, multicentre trial

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Summary

Background Increasing drug resistance limits the choice of efficacious chemotherapy against *Plasmodium falciparum* malaria in Africa. Amodiaquine still retains efficacy against *P falciparum* in many African countries. We assessed the safety, treatment efficacy, and effect on gametocyte carriage of adding artesunate to amodiaquine in three randomised trials in Kenya, Sénégal, and Gabon.

Methods We enrolled 941 children (400 in Kenya, 321 in Sénégal, and 220 in Gabon) who were 10 years or older and who had uncomplicated *P falciparum* malaria. Patients were randomly assigned amodiaquine (10 mg/kg per day for 3 days) plus artesunate (4 mg/kg per day for 3 days) or amodiaquine (as above) and placebo (for 3 days). The primary endpoints were parasitological cure rates at days 14 and 28. Analysis was by intention to treat and by an evaluability method.

Findings Both regimens were well tolerated. Six patients in the amodiaquine-artesunate group and five in the amodiaquine group developed early, drug-induced vomiting, necessitating alternative treatment. By intention-to-treat analysis, the day-14 cure rates for amodiaquine-artesunate versus amodiaquine were: 175/192 (91%) versus 140/188 (74%) in Kenya (Δ=16·7% [95% CI 9·3–24·1], p<0·0001), 148/160 (93%) versus 147/157 (94%) in Sénégal (–1·1% [–6·7 to 4·5], p=0·7), and 92/94 (98%) versus 86/96 (90%) in Gabon (8·3% [1·5–15·1], p=0·02). The corresponding rates for day 28 were: 123/180 (68%) versus 75/183 (41%) in Kenya (27·3% [17·5–37·2], p<0·0001), 130/159 (82%) versus 123/156 (79%) in Sénégal (2·9% [–5·9 to 11·7], p=0·5), and 80/94 (85%) versus 70/98 (71%) in Gabon (13·7% [2·2–25·2], p=0·02). Similar rates were obtained by evaluability analysis.

Interpretation The combination of artesunate and amodiaquine improved treatment efficacy in Gabon and Kenya, and was equivalent in Sénégal. Amodiaquine-artesunate is a potential combination for use in Africa. Further investigations to assess the potential effect on the evolution of drug resistance, disease transmission, and safety of amodiaquine-artesunate are warranted.

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Introduction

Drug-resistant *Plasmodium falciparum* malaria is a serious problem in sub-Saharan Africa. Chloroquine resistance is a major contributor to the increasing malaria-related morbidity and mortality in African children, and resistance to sulfadoxine/pyrimethamine—an inexpensive, widely used alternative—is emerging rapidly.[1–3]

New strategies are needed urgently to address the problem of declining lifespans of antimalarial drugs.[4] Combination of conventional antimalarial drugs with artemisinin derivatives (eg, oral artesunate) has been proposed as a treatment option for resistant *P falciparum*. The rationale for artemisinin-based combination therapies rests on three main arguments: (1) that drugs with independent modes of action might improve efficacy, (2) that high efficacy and gametocyte reduction might reduce malaria transmission, and (3) that resistance might be retarded because the probability of parasite resistance to both drugs is low, and because artesunate rapidly reduces the biomass of multidrug-resistant parasites, leaving few parasites to be killed by high concentrations of the companion drug.[5]

Extensive experience with artesunate-mefloquine for treating multidrug-resistant *P falciparum* malaria comes from the Thai-Burmese border—an area of low transmission of *P falciparum*. Systematic deployment of artesunate-mefloquine in this area has resulted in sustained high cure rates (95%), a reduction in *P falciparum* transmission, and increased in-vitro sensitivity of mefloquine.[6] To date, one study of an artesunate-based combination has been published from Africa. 3 days of artesunate combined with sulfadoxine/pyrimethamine was significantly better than sulfadoxine/pyrimethamine alone in children younger than 5 years in terms of resolution of symptoms, parasite reduction, and gametocyte carriage.[7] This encouraging result needs to be followed up by studies of artemisinin-based combination therapies designed to examine the effect on malaria transmission and the development of resistance in the high transmission areas of sub-Saharan Africa.

If artesunate-based combinations are to be widely introduced, clinical trials will be needed to assess their safety and efficacy. One potential partner drug is amodiaquine—a cheap 4-aminoquinoline similar to chloroquine. Amodiaquine is generally effective against chloroquine-resistant *P falciparum* infections, but efficacy varies.[8] Although serious amodiaquine-induced toxic effects have occurred with amodiaquine prophylaxis, amodiaquine is well tolerated when used as treatment.[8,9]

We report the safety, tolerability, and antimalarial efficacy of amodiaquine-artesunate and amodiaquine alone in Kenya, Sénégal, and Gabon. These studies are part of a WHO/Special Programme for Research and Training in Tropical Diseases (TDR) initiative to assess several artesunate-based combinations for treatment of falciparum malaria.

Methods

Study design, sites, and patients

These randomised, double-blind, placebo-controlled trials were done in three countries representing different forms of malaria transmission and patterns of drug resistance but where amodiaquine was expected to be efficacious. A common protocol was used with minor modifications appropriate to each study site. All study protocols were reviewed and approved by the pertinent ethics committees (Kenyan Medical Research Institute, Kenya; Academic Medical Centre, Amsterdam, Netherlands; Institut Pasteur, Dakar, Sénégal; International Foundation of the Albert Schweitzer Hospital in Lambaréné, Gabon; Medical Faculty, University of Tübingen, Germany; and WHO). Witnessed verbal or written informed consent was obtained from all parents or guardians of children.

The studies were done at four sites where *P falciparum* is the predominant species of malarial parasite. Entasopia—a village in southern Kenya—has a population of 5000. Malaria transmission is seasonal between May and July. The clinic serves mostly nomadic, rural individuals who live up to 20 km away. Amodiaquine, but not artesunate, is stocked in the clinic. Migori—a town in western Kenya—has a population of 65 000. Malaria transmission is intense and perennial. Many antimalarial drugs are available in local pharmacies. Patients were recruited from the hospital outpatient clinic. Amodiaquine efficacy (30 mg/kg total) before the study, based on a 14-day in-vivo test, was 63% (95% CI 41–81) in Entasopia, and 97% (81–100) in Migori (F Cobelens, unpublished data). Mlomp—a village in the Casamance region of southern Sénégal—has a population of 8000. Malaria transmission is seasonal (July–November), and the entomological inoculation rate is 25 per person-year. Chloroquine resistance is high: 50% in vivo in 1991, and 68% in vitro in 1997. In 1996, amodiaquine (30 mg/kg total) had a 14-day parasitological efficacy of 61%.[10,11] Artesunate and amodiaquine are not locally available. Patients, living up to 10 km away, were recruited from a small clinic. Lambaréné is a small town of 15 000 situated in west central Gabon. Malaria transmission is hyperendemic and perennial, and the entomological inoculation rate is 50 per person-year.[12] The 28-day amodiaquine (total dose 25 mg/kg) efficacy against *P falciparum* was 81% (n=63 adults) in 1994.[12,13] Amodiaquine and artesunate are both locally available.

Potential participants were medically screened. Entry criteria were: age 6 months to 10 years inclusive; weight 5 kg or more (to allow for proper dosing of artesunate); infection with *P falciparum* (1000–200 000/μL) at screening; febrile (axillary temperature ≥37·5°C or rectal temperature ≥38·5°C), or a history of fever in the preceding 24 h. Exclusion criteria included: symptoms or signs of severe malaria;[14] any danger signs (persistent vomiting, inability to drink or breastfeed or sit or stand, recent history of convulsions, lethargy, impaired consciousness);[15] serious underlying diseases (cardiac, renal, or hepatic); severe malnutrition; known allergy to study drugs; and a clear history of adequate treatment with antimalarials expected to be effective in the study area within the preceding 72 h.

Procedures

Enrolled patients were randomly assigned artesunate (Arsumax 50 mg, Sanofi-Synthélabo, France) and amodiaquine (Camoquine 200 mg, Parke-Davis, France) or placebo and amodiaquine. The randomisation code was computer-generated in blocks of 10 by Sanofi-Synthélabo. Study codes were sealed in individual envelopes and securely stored. Randomised patients were assigned a study number in numerical sequence by the investigators; both patient and investigator were unaware of study drug allocation. Artesunate or placebo tablets of identical size and colour were packaged (sequentially numbered) in four aluminium sachets (one sachet each for days 0, 1, and 2, and a spare sachet for replacement). Amodiaquine tablets were dispensed from standard medicine bottles.

Doses were artesunate 4 mg/kg bodyweight once daily for 3 days (rounded to the nearest half tablet), and amodiaquine 10 mg/kg once daily for 3 days (rounded to the nearest quarter tablet). All drug doses were administered in the clinic. Full drug doses were readministered if the patients either spat out or vomited study drugs within 1 h. Vomiting of readministered study drugs resulted in withdrawal of the patient and parenteral treatment.

Outpatient follow-up was for 28 days. Clinical and laboratory assessments were made on days 0, 1, 2, 3, 4, 7, 14, 21, 28, or days of recurrent illness. Patients or guardians were asked about drug consumption and visits to the local pharmacy since the last clinic visit. Attendance was encouraged in between these times if children became unwell. Individuals for whom treatment failed were treated with drugs appropriate for the local setting and disease severity.

Giemsa-stained blood films were read by experienced microscopists. Parasitaemia was quantified (number/μL) by a standard approximation method (40×number of parasites per 200 leucocytes on the thick film) in Sénégal and Kenya and by the Lambaréné method in Gabon.[16] A positive smear was defined as the presence of at least one asexual form seen after examining 100 thick-smear fields under ×1000 magnification. Slide quality control was done by masked rereading of 10–20% of slides, selected randomly. Discordant results were subjected to a third masked read.

Routine haematological tests (haemoglobin concentration, haematocrit, or both) and biochemical analyses (concentrations of alanine aminotransferase, aspartate aminotransferase, bilirubin, and creatinine) were done on a proportion of patients at baseline and day 28, and, in Gabon, on the day of recurrent parasitaemia. Total and differential white-cell counts were done only in Lambaréné and Entasopia. Laboratory tests were done on site except the biochemical analysis for Sénégal which was processed in France. Filter-paper blots were taken on day 0 and at the day of recurrent parasitaemia for PCR genotyping (Isocode stix, Schleicher and Schuell, Dassel, Germany). Paired PCR blots were analysed for merozoite surface proteins 1 and 2 (MSP1, MSP2), and glutamate-rich protein for patients with recurrent parasitaemia after day 16, according to previously published methods.[17]
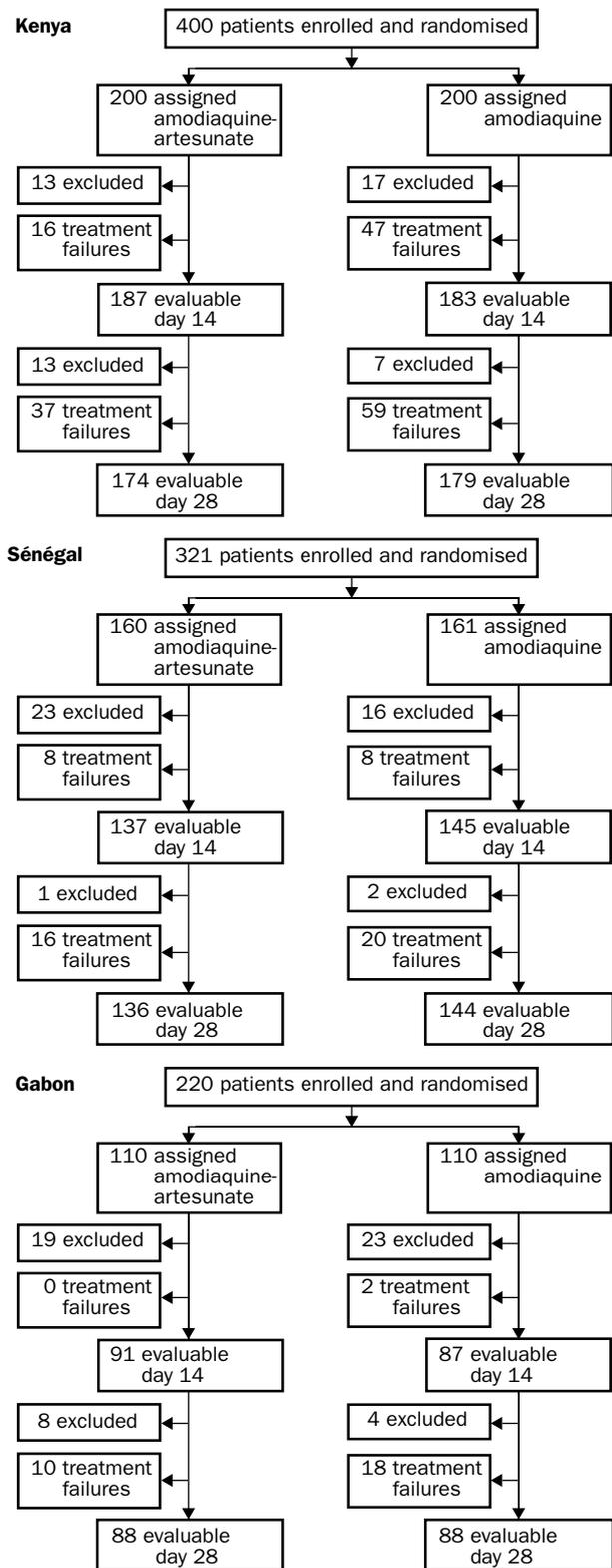
Kenya

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┌─────────────────────────────────────┐
│ 400 patients enrolled and randomised │
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200 assigned amodiaquine-artesunate	200 assigned amodiaquine
13 excluded	17 excluded
16 treatment failures	47 treatment failures
187 evaluable day 14	183 evaluable day 14
13 excluded	7 excluded
37 treatment failures	59 treatment failures
174 evaluable day 28	179 evaluable day 28

Sénégal

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┌─────────────────────────────────────┐
│ 321 patients enrolled and randomised │
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160 assigned amodiaquine-artesunate	161 assigned amodiaquine
23 excluded	16 excluded
8 treatment failures	8 treatment failures
137 evaluable day 14	145 evaluable day 14
1 excluded	2 excluded
16 treatment failures	20 treatment failures
136 evaluable day 28	144 evaluable day 28

Gabon

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┌─────────────────────────────────────┐
│ 220 patients enrolled and randomised │
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110 assigned amodiaquine-artesunate	110 assigned amodiaquine
19 excluded	23 excluded
0 treatment failures	2 treatment failures
91 evaluable day 14	87 evaluable day 14
8 excluded	4 excluded
10 treatment failures	18 treatment failures
88 evaluable day 28	88 evaluable day 28

Figure 1: Trial profile
Numbers refer to evaluability analysis (PCR uncorrected). Exclusions from evaluability analysis on day 14 (respectively amodiaquine-artesunate, Kenya; amodiaquine, Kenya; amodiaquine-artesunate, Sénégal; amodiaquine, Sénégal; amodiaquine-artesunate, Gabon; amodiaquine, Gabon) were for the following reasons. Entry criteria not met: 2, 3, 18, 11, 3, 1; wrong dosage: 3, 2, 1, 2, 2, 2; study-drug course not completed: 0, 1, 0, 1, 0, 1; absent day 14: 0, 3, 0, 1, 5, 5; lost to follow-up: 7, 8, 0, 1, 7, 5; adverse events: 0, 0, 4, 0, 2, 5; other antimalarials: 1, 0, 0, 0, 0, 4. The respective exclusions on day 28 were for the following reasons. Lost to follow-up: 12, 6, 1, 1, 6, 4; adverse events: 0, 0, 0, 1, 0, 0; other antimalarials: 1, 1, 0, 0, 2, 0.

Outcomes

The two prospectively defined primary efficacy outcomes were the parasitological cure rates on days 14 and 28 at the individual study sites—ie, initial clearance of parasitaemia that was sustained to day 14 and day 28. The criteria for treatment failure were: development of severe malaria or danger signs; parasitaemia at 48 h at least as high as the parasite count at day 0; parasitaemia on day 3 at least 25% of the count on day 0 plus fever (Kenya and Sénégal), or parasitaemia at any time from day 3 to day 6 at least 25% of the day 0 count (Gabon); parasitaemia on day 7; and initial parasite clearance followed by recurrence up to day 28. Drug safety and tolerability were assessed clinically and by laboratory tests. An adverse event was defined as a sign, symptom, intercurrent illness, or abnormal laboratory value not present on day 0 but which occurred during follow-up, or was present on day 0 but became worse during follow-up. The relation between the adverse event and study drug was judged by the clinicians in the field and designated as definite, probable, possible, unlikely, not related, or unknown. A serious adverse event was defined as lethal, life threatening, or requiring hospital admission.[18]

The day-28 cure rate was adjusted on the basis of the PCR genotyping results of paired samples for patients with recurrent parasitaemia from days 17 to 28. Patients with matching genotypes on day 0 and the day of recurrence were classified as having treatment failure; those with mismatching genotypes were classed as cured. Patients with missing PCR data were regarded as having treatment failure. PCR data were not available for Sénégal.

The secondary outcomes were: the proportions of patients who were (1) aparasitaemic, and (2) afebrile at 24, 48, and 72 h; the proportions of patients with gametocytes during follow-up; and the fractional change in haemoglobin or haematocrit, comparing day 28 with day 0.

Statistical analysis

Sample sizes were calculated for each site on the basis of recent or presumed cure rates for amodiaquine alone (CR_A) or combined with artesunate (CR_{AA}) with a two-sided α of 0·05 and a power of 80%. Allowing for loss to follow-up (10–20%), the sample sizes per group were 200 for Kenya and Sénégal (CR_A=85%, CR_{AA}=95%), and 110 for Gabon (CR_A=80%, CR_{AA}=95%).

Data were double-entered, validated (EpiInfo version 6.04b, Centers for Disease Control and Prevention, Atlanta, GA, USA), and analysed with Stata version 6.0 (Stata Corporation, TX, USA), and JMP version 4.0 (SAS Institute, Cary, NC, USA). Primary outcomes for each study were analysed separately by intention to treat and by an evaluability method.

The intention-to-treat analysis excluded patients who were randomised in error (did not meet entry criteria—eg, had severe anaemia or a significant concurrent illness). Other violations of entry criteria—eg, wrong age—were not excluded. Withdrawals due to an adverse event, or use of another drug with antimalarial activity, were regarded as treatment failures. The evaluability analysis excluded all violations of entry criteria and all study withdrawals. Both analyses excluded patients lost to follow-up. Patients who were absent on day 14 but continued follow-up to day 28 were excluded from the day-14 analysis but included in the day-28 analysis.

Binary data were analysed by estimation of differences in proportions with corresponding 95% CIs. Exact 95% CIs were calculated when the proportions were close to zero or one (eg >95% cure rate), otherwise normal estimations were used.[19] Student's unpaired and paired t tests were used to assess differences between and within groups in mean

	Kenya		Sénégal		Gabon	
	Amodiaquine-artesunate (n=200)	Amodiaquine (n=200)	Amodiaquine-artesunate (n=160)	Amodiaquine (n=161)	Amodiaquine-artesunate (n=110)	Amodiaquine (n=110)
Male/female	105/95	108/92	76/84	89/72	57/53	58/52
Age (years)	2·9 (2·4)	2·7 (2·3)	8·1 (2·6)	7·3 (2·6)	5·5 (2·3)	6·2 (2·3)
Weight (kg)	12·1 (4·4)	11·9 (4·6)	25·1 (8·2)	22·5 (7·5)	17·8 (5·1)	18·7 (5·1)
Temperature (ºC)	38·4 (1·2)	38·4 (1·2)	38·5 (1·0)	38·6 (0·9)	37·5 (1·1)	37·7 (1·2)
Parasite count/µL	31 640 (1240–392 080)	30 720 (1160–416 800)	41 760 (1400–426 680)	51 400 (1520–458 640)	26 231 (2000–180 000)	20 296 (2200–196 530)
Haemoglobin (g/L)	93 (19)	93 (20)	..	..	99 (17)	101 (16)
Haematocrit (%)	..	..	37 (5·2)	36 (5·6)	32 (5·1)	32 (4·8)
Leucocyte count (×10⁹/L)	9·8 (3·7)	9·4 (3·9)	..	..	8·0 (3·0)	7·7 (3·0)
ALT (IU/L)	22 (19)	19 (11)	12 (9)	15 (12)	29 (25)	32 (62)
Creatinine (µmol/L)	..	..	68 (13)	64 (12)	24 (32)	38 (29)
Total bilirubin (µmol/L)	23 (13)	23 (11)	12 (6)	13 (8)	17 (15)	13 (11)

Data are mean (SD) except for parasite count (geometric mean [range]). ALT=alanine aminotransferase.

Table 1: **Demographic, clinical, and laboratory characteristics of patients at enrolment**

values for normally distributed laboratory data; corresponding parametric tests were used as appropriate. An aggregate meta-analysis (www.cochrane.org/cochrane/revman.htm, accessed April 2, 2002) based on the intention-to-treat data was used to combine the results from the three trials.

Role of the funding source
WHO/TDR were involved in all aspects of the trials (design, conduct, analysis, interpretation, and writing), but Sanofi-Synthélabo and Parke-Davies, who provided the drugs, had no such role.

Results
Patients' characteristics
941 patients (470 amodiaquine-artesunate, 471 amodiaquine alone) were randomised between the following times: January, 1999–January, 2000 in Gabon (n=220), July, 1999–December, 1999 in Sénégal (n=321), and June, 1999–June, 2000 in Kenya (Entasopia n=160, and Migori n=240). Figure 1 shows the flow of patients through each trial; the numbers refer to the evaluability analysis. Patients at each centre in the two treatment groups had similar demographic, clinical, and laboratory characteristics at enrolment (table 1). The proportions of children younger than 5 years differed: 320 of 400 (80%) in Kenya, 90 of 220 (41%) in Gabon, and 42 of 321 (13%) in Sénégal. The study in Sénégal did not meet its target number of patients because recruitment tailed off in parallel with the malaria season.

Intention-to-treat analysis
Five ineligible patients (three assigned amodiaquine-artesunate, two assigned amodiaquine) were excluded from

the analysis because of a history of adequate treatment, a negative day-0 slide, inadvertent allocation of an extra study number (amodiaquine-artesunate), danger signs, or the presence of another significant illness (amodiaquine). Losses to follow-up were 35 (15 amodiaquine-artesunate, 20 amodiaquine) by day 14, and a further 34 (19 amodiaquine-artesunate, 15 amodiaquine) by day 28. Absentees on day 14 numbered 14 (six amodiaquine-artesunate, eight amodiaquine). Within the first week, there were 50 failures (15 amodiaquine-artesunate, 35 amodiaquine): nine with danger signs or severe malaria (four amodiaquine-artesunate, five amodiaquine), five with day-2 parasitaemia at least as high as at day 0 (five amodiaquine), 20 with day-7 parasitaemia (four amodiaquine-artesunate, 16 amodiaquine), 11 with adverse events (six amodiaquine-artesunate, five amodiaquine), and five who consumed a drug with antimalarial activity (one amodiaquine-artesunate, four amodiaquine).

At day 14, cure rates with amodiaquine-artesunate were significantly higher than for amodiaquine alone in Kenya and Gabon, but were similar between the groups in Sénégal (table 2). Cure rates decreased during follow-up in all sites. Amodiaquine-artesunate was significantly more efficacious than amodiaquine alone in Kenya and Gabon, but not in Sénégal. The PCR-adjusted rates are also shown in table 2. All samples from Gabon were genotyped and classified as either reinfection or recrudescence. In Kenya, 55 were missing and one PCR pair was not interpretable; they were classified as failures (19 amodiaquine-artesunate, 37 amodiaquine).

Evaluability analysis
111 children were excluded from analysis by day 14 and a further 35 by day 28 (figure 1); 15 of the 111 exclusions

	Amodiaquine-artesunate	Amodiaquine	Δ (95% CI)	p
Kenya				
Cure rate day 14	175/192 (91%)	140/188 (74%)	16·7% (9·3 to 24·1)	<0·0001
Cure rates day 28				
PCR uncorrected	123/180 (68%)	75/183 (41%)	27·3% (17·5 to 37·2)	<0·0001
PCR corrected*	144/180 (80%)	98/183 (54%)	26·4% (17·2 to 35·7)	<0·0001
Sénégal				
Cure rate day 14	148/160 (93%)	147/157 (94%)	–1·1% (–6·7 to 4·5)	0·69
Cure rates day 28				
PCR uncorrected	130/159 (82%)	123/156 (79%)	2·9% (–5·9 to 11·7)	0·52
PCR corrected*	..	..	..	..
Gabon				
Cure rate day 14	92/94 (98%)	86/96 (90%)	8·3% (1·5 to 15·1)	0·016
Cure rates day 28				
PCR uncorrected	80/94 (85%)	70/98 (71%)	13·7% (2·2 to 25·2)	0·02
PCR corrected*	85/94 (90%)	77/98 (79%)	11·9% (1·8 to 21·9)	0·02

*Missing PCR data=failures.

Table 2: **Intention-to-treat analysis**

	Amodiaquine-artesunate	Amodiaquine	Δ (95% CI)	p
Kenya				
Cure rate day 14	171/187 (91%)	136/183 (74%)	17·1% (9·6 to 24·6)	<0·0001
Cure rates day 28				
PCR uncorrected	121/174 (70%)	73/179 (41%)	28·8% (18·8 to 38·7)	<0·0001
PCR corrected*	141/174 (81%)	96/179 (54%)	27·4% (18·1 to 36·7)	<0·0001
Sénégal				
Cure rate day 14	129/137 (94%)	137/145 (95%)	−0·3% (−5·7 to 5·1)	0·91
Cure rates day 28				
PCR uncorrected	112/136 (82%)	116/144 (81%)	1·8% (−7·3 to 10·9)	0·70
PCR corrected*	..	..	..	..
Gabon				
Cure rate day 14	91/91 (100%)	85/87 (98%)	2·3% (−0·9 to 5·4)	0·15
Cure rates day 28				
PCR uncorrected	78/88 (89%)	68/88 (77%)	11·4% (0·4 to 22·3)	0·043
PCR corrected*	83/88 (94%)	75/88 (85%)	9·1% (0·2 to 17·9)	0·044

*Missing PCR data=failures.

Table 3: **Evaluability analysis**

were absent on day 14 but continued follow-up and are included in the day-28 analysis. Age exceeding 10 years at enrolment accounted for the high number of exclusions in Sénégal. Treatment outcomes were evaluable for 830 (88%) patients at day 14 and 809 (86%) at day 28. Within the first week, there were 33 failures (eight amodiaquine-artesunate, 25 amodiaquine): nine with danger signs or severe malaria, five with day-2 parasitaemia at least as high as at day 0, and 19 with day-7 parasitaemia (four amodiaquine-artesunate, 15 amodiaquine).

At day 14, cure rates with amodiaquine-artesunate were similar to those with amodiaquine in Sénégal and Gabon, but significantly higher in Kenya. The cure rates decreased during follow-up in all sites. Amodiaquine-artesunate was significantly more efficacious than amodiaquine alone in Kenya and Gabon, but not in Sénégal (table 3). The PCR-adjusted rates are also shown in table 3. In Kenya, 45 samples were missing and one PCR pair was not interpretable; all were classified as failures (12 amodiaquine-artesunate, 34 amodiaquine).

Aggregate meta-analysis
Combination of the cure rates on days 14 and 28 (unadjusted for PCR) for all three sites gave fixed odds ratios of 2·5 (95% CI 1·6–3·8) and 2·2 (1·6–3·0), respectively, in favour of the combination over amodiaquine alone.

Parasite clearance and gametocyte carriage
In all three studies, amodiaquine-artesunate substantially accelerated the clearance of parasites compared with amodiaquine on days 1, 2, and 3 (figure 2). By day 1, fever clearance rates were high in all groups, but were significantly higher for amodiaquine-artesunate in Kenya (191/199 [96%] *vs* 164/196 [84%], p<0·0001] and Gabon (103/106 [97%] *vs* 90/105 [86%], p=0·002); corresponding results for Sénégal were 150/160 (94%) versus 154/159 (97%), p=0·7.

At baseline, gametocyte carriage rates were low. Gametocyte carriage initially rose in the amodiaquine groups in all three sites, but decreased by days 21 and 28. Amodiaquine-artesunate significantly reduced gametocyte carriage compared with amodiaquine only on day 7 (14/164 [9%] *vs* 30/171 [18%], −9·0% [−1·8 to −16·1]) and day 14 (8/174 [5%] *vs* 18/157 [12%], −6·9% [−1·0 to −12·7]) in Kenya (figure 3).

Adverse events
Amodiaquine and amodiaquine-artesunate were well tolerated. Early drug-induced vomiting necessitating alternative treatment occurred in 11 patients: six on

amodiaquine-artesunate (four on day 0, two on day 1), and five on amodiaquine (two on day 0, two on day 1, one on day 2). The reporting of any symptom (weakness, headache, dizziness, anorexia, nausea, vomiting, abdominal pain, diarrhoea) within the first week and during follow-up was similar between the groups, as was the total number of reported adverse events: 165 in the amodiaquine-artesunate group versus 133 in the amodiaquine only group. Nine patients had dermatological complaints: eight
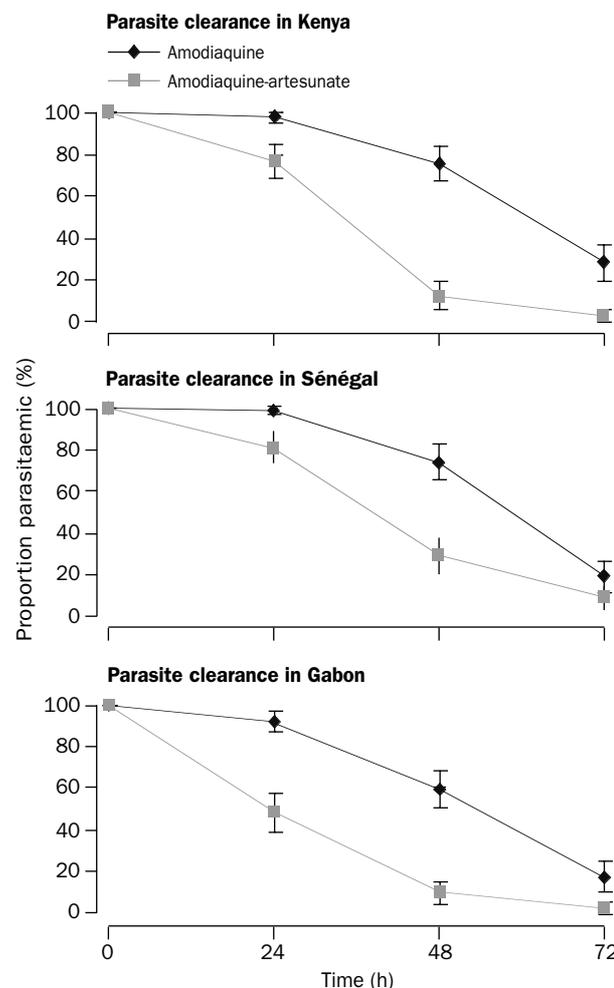
Figure 2: **Proportion of children with patent asexual *Plasmodium falciparum* parasitaemia during first 3 days of treatment in Kenya, Sénégal, and Gabon**
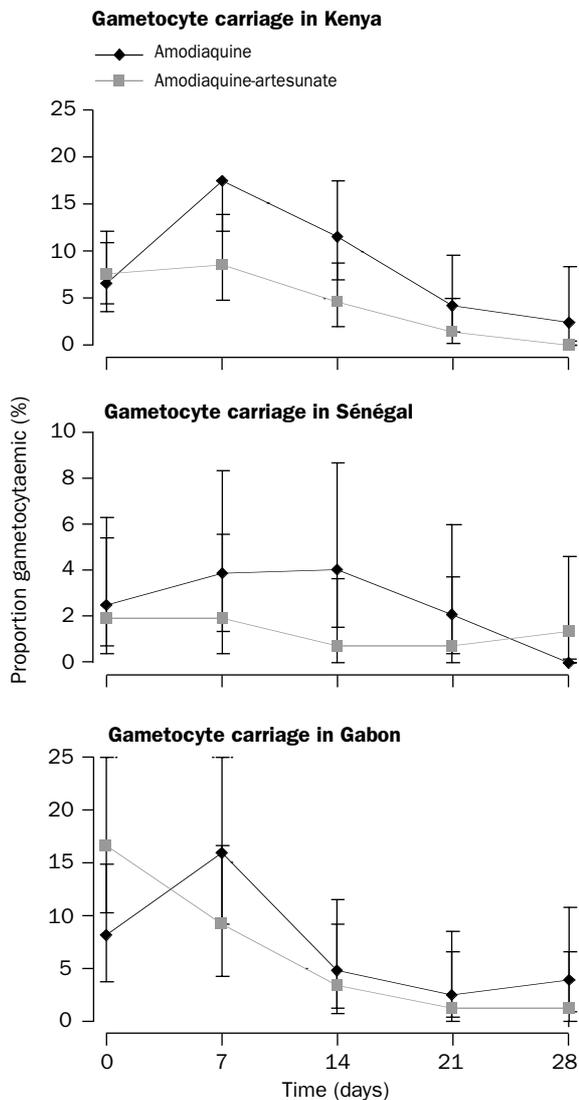Vertical lines indicate 95% CIs.

Figure 3: Proportion of children with gametocytes at baseline and follow-up in Kenya, Sénégal, and Gabon
Vertical lines indicate 95% CIs.

(amodiaquine) reported itching, and one (amodiaquine-artesunate) developed an itchy rash.

There was one death (amodiaquine) and 13 other serious adverse events (seven amodiaquine-artesunate, six amodiaquine). In Kenya, three patients had convulsions, two had pneumonia, two anaemia, and one meningitis; in Sénégal, one had convulsions; and in Gabon, one had asthma, one convulsions, one vomiting on day 0, and one gastroenteritis. Apart from the vomiting on day 0, all serious adverse events were regarded as unrelated to study drugs.

The death occurred in a four-year-old Kenyan boy from Migori who received amodiaquine alone. He became acutely breathless with foaming at the mouth 6 h after the first dose of amodiaquine. He died in hospital 6 h after the development of dyspnoea. The cause of death was unclear (no necropsy was done), but the history was consistent with acute pulmonary oedema or convulsions. He was classified as having treatment failure due to severe malaria.

Mean values of routine haematological and biochemical variables were similar in the two groups at each timepoint (details not shown). By day 28, cured patients had similar increases in mean haemoglobin concentrations compared with baseline: 15 g/L (SD 19, n=184) for amodiaquine-artesunate versus 14 g/L (20, 133) for amodiaquine alone. Concentrations of liver enzymes (n=192) tended to decrease during follow-up, but mean changes were not significantly different compared with baseline between the groups (details not shown). Changes in serum creatinine values were also unremarkable (n=201). By day 28, 153 (n=140 Gabon, n=13 Kenya) patients had paired day 28/day 0 white-cell counts. Of these, nine children (6% [95% CI 3–11]) developed neutropenia (absolute neutrophil count <1000/μL); their neutrophil counts ranged from 306 to 900/μL (median 780). Three were on amodiaquine-artesunate and six were amodiaquine recipients; all had normal neutrophil counts on day 0. There was a significant decline in mean neutrophil counts, comparing each timepoint with baseline, with no differences between the two treatment groups (details not shown). Mean total white-cell counts and mean changes in white-cell counts compared with day 0 were unremarkable between the two groups (details not shown).

Discussion

For malaria-control programmes to benefit fully from effective antimalarial drugs, their regimens should be highly efficacious, of short duration, well tolerated, and cheap. An added bonus would be the propensity to reduce transmission and limit the development of resistance—a possibility that might be realised with the artemisinin derivatives. Indeed, artesunate-based combinations are currently being evaluated to assess their role in developing new antimalarial drug policies for African countries.

We assessed amodiaquine-artesunate for treatment of paediatric falciparum malaria in three African countries that have different patterns of malaria transmission and rates of chloroquine and amodiaquine resistance. A common protocol was used to allow for a meta-analysis of individual patients' data. This meta-analysis is not yet complete but will be reported shortly.

We analysed the efficacy data by means of an intention-to-treat and an evaluability method. Within the context of a controlled trial, an intention-to-treat analysis, by including predefined, non-parasitological failures (eg, drug-induced vomiting), attempts to answer the general, programmatic question of whether amodiaquine-artesunate will make the patient better.[20] An evaluability analysis addresses clinical and parasitological failure (resistance) after the administration of the correct drug dose to a well defined sample of patients, consistent with the WHO definition of drug resistance.[21]

Overall, these three trials have shown that the combination was well tolerated and efficacious. By day 14, cure rates exceeded 90% in all three countries. The rates were lower by day 28. In Sénégal, the combination had equivalent efficacy to monotherapy at both day 14 and day 28, which was an unexpected finding and difficult to explain, given its better pharmacodynamic effect on parasite reduction. By contrast, the combination significantly improved efficacy in Kenya and Gabon. A greater effect was seen in Kenya—the country with the highest rate of amodiaquine treatment failures. Although these treatment failures might be partly explained by the young age and relative lack of acquired immunity of the Kenyan children, this degree of resistance in a key vulnerable group is of concern and casts doubt on the use of amodiaquine-artesunate in Kenya. The effect of amodiaquine-artesunate on gametocyte carriage was also most beneficial in Kenya,

again consistent with its high level of amodiaquine resistance.[22]

Amodiaquine-artesunate was well tolerated. Early on, drug-induced vomiting requiring retreatment was confined to a very small number of patients in both groups. Two serious complications of amodiaquine described in published studies are hepatitis and neutropenia. Risk estimates, based on weekly amodiaquine prophylaxis, are 1 in 15 650 and 1 in 2000 for these two disorders, respectively.[23] We did not see any cases of clinical hepatitis, but our sample size was small. There was, however, a decline in serial neutrophil counts in 60% of children from both study groups, and a small number who developed neutropenia without apparent clinical ill effects. Published data on amodiaquine-related neutropenia and hepatitis are few. Of pertinence are reports of a case of symptomless hepatitis in a normal volunteer after two doses of amodiaquine and artesunate, and a decline in mean absolute neutrophil counts after amodiaquine, sulfadoxine/pyrimethamine, and amodiaquine-sulfadoxine/pyrimethamine combined.[8,24,25] Other studies have not reported changes in the total white-cell or neutrophil counts.[10,26] We cannot definitively ascribe the cause of neutropenia in our patients to amodiaquine because malaria itself could be a factor. Nevertheless, this finding calls for further work to assess the safety and to define the risk:benefit ratio of repeated amodiaquine or amodiaquine-artesunate use.

Experience with other artemisinin-based combinations comes from Africa and Asia. Parasitological cure rates, some PCR-adjusted, have ranged from 93 to 99% in young African children (artesunate/ sulfadoxine-pyrimethamine, artemether/lumefantrine); Thai children and adults (artesunate/mefloquine, artemether/lumefantrine); and Indian adults (artemether/ lumefantrine). However, artemether/lumefantrine had a comparatively modest day-14 cure rate of 87% in 110 Tanzanian children aged 1–5.[7,27–31] Our day-14 results with amodiaquine-artesunate are consistent, but only the day-28 result from Gabon exceeded 90%.

For African countries that are considering a change of their current first-line antimalarial drug, amodiaquine-artesunate is an option. Cost, access, and local efficacy data are also fundamental elements to consider before amodiaquine-artesunate or other drug combinations are implemented as policy. Longitudinal studies should now address the general question of how best to deploy artemisinin-based combinations, and the safety of amodiaquine-artesunate and its potential effect on the development of drug resistance and transmission.

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Clinical picture

Scapular calcinosis

Everett H Allen

A 48-year-old woman presented with 3-week history of progressive swelling over both scapulae. She had dermatomyostis complicated by perineal calcinosis with skin breakdown and infection. One such infection culminated in necrotising fasciitis that required extensive debridement and left her wheelchair-bound. On physical examination, a large fluctuant non-tender mass was noted over the left distal scapula. A similar mass with a more heterogeneous texture was noted in the right posterior axillary line. A scapular radiograph (figure) demonstrated lobular soft tissue calcification extending from the dorsal aspect of the scapula, surrounding the tip of the scapula, and continuing between the scapula and chest wall.

Aspiration of the mass yielded 75 mL of white fluid with a white blood cell count of 2500/mm³ with 69% macrophages. Polarised microscopy of the fluid showed acellular amorphous debris without birefringence. Calcinosis deposits in scleroderma are most common on the extremities and over bony eminences, particularly at points of pressure, while those in dermatomyositis may involve subcutaneous tissues and deeper structures.

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