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Efficacy of artesunate plus chloroquine for uncomplicated malaria in children in Sao Tome and Principe: a double-blind, randomized, controlled trial

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Abstract

We conducted a double-blind, randomized, placebo-controlled trial in Sao Tome and Principe to investigate the safety, tolerability and efficacy of chloroquine (CQ) combined with artesunate (AS) over CQ monotherapy. Four hundred children, aged 6–59 months, with acute uncomplicated *Plasmodium falciparum* malaria were randomized to receive a standard dose of CQ (25 mg/kg bodyweight) over 3 d or CQ + AS (4 mg/kg bodyweight) daily for 3 d. Children were followed-up for 28 d. The combined treatment was well tolerated and there were no serious drug-related adverse events. By day 2 parasite clearance was significantly faster for children treated with CQ + AS compared with CQ alone (29/194 [14.9%] vs. 168/190 [88.4%] still parasitaemic, $P < 0.0001$). Day 14 parasitological failure rates were 153/191 (80.1%) for CQ alone compared with 32/193 (16.6%) in the CQ + AS group (odds ratio [OR] = 20.2, 95% CI 11.7–35.4, $P < 0.001$). Corresponding clinical failure rates were 128/191 (67.0%) and 12/193 (6.2%) (OR = 30.6, 95% CI 15.3–62.7, $P < 0.001$). By day 28 the parasitological failure rates (new infections excluded) were 155/191 (81.1%) in the CQ group and 63/194 (32.4%) in the CQ + AS group (OR = 8.9, 95% CI 5.4–14.7, $P < 0.001$). Symptoms resolved faster in children who received AS. They were also less likely to be gametocytaemic after treatment. The combination treatment was well tolerated and considerably improved treatment efficacy. However, the current levels of CQ resistance preclude its use in Sao Tome where CQ should be abandoned as first-line drug. However, CQ + AS may be an option in areas where CQ resistance is lower.

Keywords: malaria, *Plasmodium falciparum*, combination therapy, artesunate, chloroquine, Sao Tome and Principe

Introduction

Over the past 20 years, malaria treatment and control have been undermined by the emergence of resistance to widely used antimalarial drugs (WHO, 1993). *Plasmodium falciparum* resistance to chloroquine (CQ), which was first documented in East Africa in 1978, is now widespread in sub-Saharan Africa and has resulted in increased malaria mortality (Trape, 2001) and morbidity, e.g. only transient clinical improvement and poor haematological recovery (Bloland *et al.*, 1993). Chloroquine is reportedly still relatively efficacious in some West African countries such as Mali (Djimde *et al.*, 2001) and Burkina Faso (Coulibaly, 2000), although resistance is likely to increase and will necessitate a change in drug policy.

The artemisinin derivatives cause a rapid and substantial decrease of the parasite load and their use in combination with other antimalarial drugs has been recommended (WHO, 2001). The combination of artesunate (AS) with another antimalarial drug such as mefloquine (Nosten *et al.*, 1994), sulfadoxine–pyrimethamine (SP) (von Seidlein *et al.*, 2000), and amodiaquine (Adjuik *et al.*, 2002) has been shown to be safe, well tolerated, and highly efficacious. It is important to establish whether other antimalarial drugs can be safely and effectively combined with AS in sub-Saharan Africa. We therefore investigated the safety, tolerability and efficacy of CQ + AS compared with CQ alone in children from Sao Tome with acute uncomplicated *P. falciparum* malaria in a double-blind, randomized, controlled study.

Methods

Study site

The study was conducted on the islands of Sao Tome and Principe, at the Guadalupe Health Centre (Lobata District) and at the Delegacia de Saúde Health Centre (ex-Mission d'Eradicação de Paludismo, MEP) in the capital city, Sao Tome; both health centres are situated in the north of the island about 10 km from each other. The island of Sao Tome is situated in the Gulf of Guinea, approximately 280 km from Gabon on the West African coast. The climate is equatorial, hot and humid, although the northern part of the island receives less rainfall (1500 mm/year) than the rest of the island (7000 mm/year). The total population numbers about 130 000 people; one-third live in the capital city. Malaria transmission is stable with seasonal peaks. The major vector is *Anopheles gambiae sensu stricto*. All 4 human malaria species are present but *P. falciparum* is predominant and is the most common cause of death among children aged < 5 years (Baptista *et al.*, 1997). The prevalence of treatment failure among children aged < 11 years, using WHO criteria (1996), was estimated at 7.6% in 1995, 16.1% in 1996, and 20% in 1997 (Centro Nacional de Endemias, Sao Tome and Principe, documentos técnicos, 1995–1997). At the time of the study CQ was the recommended first-line treatment for uncomplicated malaria.

Patients

Children aged 6–59 months and with fever (body temperature ⩾ 37.5 °C) or history of fever in the previous 24 h and a presumptive diagnosis of 'clinical malaria' were screened for malaria infection. Children weighing ⩾ 5 kg with a *P. falciparum* monoinfection and a parasite density of 10 000–100 000 parasites/μL were recruited if a parent or guardian gave informed consent. Children were excluded if they had (i) danger signs (unable to drink or breastfeed, vomiting more than twice in 24 h, recent history of convulsions, unconscious state or unable to sit or stand); (ii) signs of severe malaria (WHO, 2000); (iii) a packed cell volume (PCV) < 15%; (iv) clear history of adequate malaria treatment in the preceding 72 h; and (v) any evidence of chronic disease.

Study design

All enrolled children received a standard dose (25 mg/kg bodyweight over 3 d) of CQ (chloroquine

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phosphate, International Dispensary Association, Amsterdam, Holland) and were randomized to receive either placebo or AS (Sanofi/Synthélabo, Gentilly, France) at a dose of 4 mg/kg bodyweight/d for 3 d. The AS and placebo were identical in appearance, packaged individually, and labelled with a randomization number for each child in blocks of 12. All doses were given under direct supervision. Each child received paracetamol (10 mg/kg/dose) when needed and the parents were instructed to give paracetamol when the child had fever. Children were observed for 1 h for vomiting and a replacement dose was given if necessary. Investigators, children and their parents or guardians remained 'blinded' to the treatment allocation throughout the study. Patients (parent/guardian) were asked to return to the clinic 24 and 48 h later for drug administration and for scheduled tests at 72 h and at days 7, 14, 21, and 28. If the patient did not report for scheduled visits every effort was made by the nurses to locate them at their home address. Parents were encouraged to return to the health centre any time the child was unwell. A blood slide for parasitaemia was prepared at days 0–3 and at days 7, 14, 21, and 28. The PCV was measured at days 0, 7, 14, and 28. A blood sample for liver function tests (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), total bilirubin and creatinine was collected at days 0, 7, and 28 from the first 75 patients. Filter-paper blood blots were collected for genotyping on days 0, 7, 14, 21, and 28 or any day of recurrent parasitaemia. Sulfadoxine–pyrimethamine was given in case of treatment failure: (i) parasite density at day 2 > day 0; (ii) parasite density at day 3 ⩾ 25% of parasite density at day 0 and patient unwell (according to the evaluation of the treating physician); (iii) parasite density at day 4 ⩾ 25% of parasite density at day 0; (iv) parasitaemia at days 7; or (v) recurrent parasitaemia (positive blood slide following a negative one). Parenteral quinine was given as clinically indicated. An adverse event (AE) was defined as any new symptom or an exacerbation of old symptoms occurring after the start of treatment.

The Ministry of Health of Sao Tome and Principe reviewed and approved the study.

The study was also approved by the ethics review board of the Prince Leopold Institute of Tropical Medicine (Antwerp, Belgium) and by the World Health Secretarial Committee for Research Involving Human Subjects.

Laboratory methods

Thick blood films were Giemsa-stained. Parasite density was determined on the basis of the number of parasites per 200 leucocytes on a thick film assuming a total leucocyte count of 8000/μL. If gametocytes were seen, the gametocyte count was extended to 1000 leucocytes. Each slide was read 'blind' by the same 2 experienced microscopists and discrepancies were reviewed. The PCV was measured by microhaematocrit centrifugation. Biochemical parameters were measured using a Piccolo Portable Blood Analyzer (Abaxis Inc., Sunnyvale, CA, USA).

Blood samples on filter-paper were used for analysis of merozoite surface proteins 1 and 2 (MSP1 and MSP2) and glutamine rich protein (GLURP). If the child had a second episode of parasitaemia, samples from the first and second episodes were assayed. DNA was purified as described previously (Irion *et al.*, 1998). A nested polymerase chain reaction (PCR) was used for the analysis of 3 polymorphic genetic markers from *P. falciparum*: the 3 sequence families of the MSP1 block 2 repeat region, the 2 sequence families of the MSP2 repeat region, and the RII region of GLURP. A recrudescent infection was defined as one that showed a complete match in allelic size for all the genes *MSP1*, *MSP2*, and *GLURP* between the first and second samples. If any clone of a polyclonal primary infection was detected during a second episode it was considered a recrudescence.

Statistical methods

Data were double-entered and validated using Epi Info 6.4b (CDC, Atlanta, GA, USA). Analyses were made with SPSS for Windows release 10.0.05 (SPSS Inc., Chicago, IL, USA). The parasitological failures at day 14 and day 28 were the primary efficacy outcomes. Children were considered failures if they had received rescue treatment by day 14 or were parasitaemic on day 14. All children for whom an outcome was known were included in the analysis. Children were considered failures by day 28 if they received rescue treatment on or before day 28, or if they were parasitaemic on day 28. In a secondary analysis, children were considered not to be treatment failures if their parasitaemia on day 28 was caused by a new rather than recrudescent infection according to the PCR genotyping. Children who received rescue treatment before or were parasitaemic and febrile (body temperature ⩾ 37.5 °C) at day 14 were considered as clinical failures. Chi-squared analysis was used to compare the failure rates between the 2 groups and a 95% CI was computed for the corresponding odds ratio (OR). Laboratory data were analysed using the *t* test or paired *t* test.

Results

Four hundred children meeting the entry criteria were recruited out of 2911 screened between May and December 1999 and 200 were allocated to each treatment group. Four children in the CQ group and 1 in the CQ + AS group had a parasite density at day 0 slightly over 100 000/μL (102 000–116 440), while 1 in the CQ + AS group had a density of 9800/μL. These children were included in the analysis. At enrolment the groups had similar demographic and clinical characteristics (Table 1). Treatment outcome by day 14 was known for 384 (96%) children; 14 were withdrawn by their parents/guardians and 2 came to the clinic only at day 21. Outcome by day 28 was known for 385 (96%) children; 1 child was lost at follow-up on day 28 and 14 children were withdrawn before day 14.

The CQ + AS combination was well tolerated. There were no serious, treatment-related AEs. Children presented most frequently with headache, anorexia, vomiting, and diarrhoea. By days 2 and 3, symptom resolution was significantly more rapid in children who received CQ + AS. The proportion of children with any symptom decreased from 99.5% in both groups at day 0 to 68% (111/164) in the CQ group and 53% (103/194) in that of CQ + AS ($P = 0.005$) at day 3. An AE was recorded between days 0 and 7 in 42 (21%) children in the CQ group and in 26 (13%) in the CQ + AS group ($P = 0.03$). The most frequent AEs were convulsions, diarrhoea, and vomiting. Convulsions occurred only until day 4 and were more frequent in the CQ alone (17 children) group than in the CQ + AS (4 children) group. The study treatment was immediately discontinued and quinine administered. Between days 8 and 28, an AE was recorded in 4 (6.1%) of 66 children in the CQ group and in 20 (10.8%) of 186 children in the CQ + AS group ($P = 0.26$). The most common AEs were diarrhoea, cough, and acute otitis media. All reported AEs were considered unrelated to the study drugs except 2 of the 200 (1%) CQ recipients who developed pruritus.

No major difference in the raised concentrations of AST (> 38 IU/L) and ALT (> 47 IU/L) at days 0 and 7 were found between the 2 treatment groups. In the CQ group 5/36 (13.9%) children had raised bilirubin levels at day 0. All had resolved by day 7. All children had normal creatinine concentrations before and after treatment.

By day 7, 122 (63.8%) of 191 children in the CQ group and 6 (3.1%) of 193 in the CQ + AS group were

Table 1. Baseline characteristics of two study groups of children with acute uncomplicated *Plasmodium falciparum* malaria, Sao Tome and Principe, May–December 1999

	Chloroquine alone ($n = 200$)	Chloroquine + artesunate ($n = 200$)
Demographic characteristics		
Mean (range) age (months)	28.5 (6–59)	27.0 (6–59)
Males/females	105/95	88/112
Clinical characteristics		
Mean (range) weight (kg)	11.5 (5.5–22.0)	11.3 (5.7–20.0)
Mean (range) temperature (°C)	37.7 (35.9–40.4)	37.7 (35.9–40.2)
Temperature ≥ 37.5°C	115 (57.5%)	114 (57.0%)
Hepatomegaly	129 (64.5%)	123 (61.5%)
Splenomegaly	189 (94.5%)	188 (94.0%)
Geometric mean (range) asexual *P. falciparum*/μL	38 572 (10 100–116 440)	36 579 (9800–102 000)
Gametocyte rate	6 (3.0%)	5 (2.5%)
Mean (SD) packed cell volume	31.2 (7.8) ($n = 197$)	32.2 (8.3) ($n = 199$)

parasitaemic or had already received rescue treatment (OR = 55.1, 95% CI 22.1–158.2, $P < 0.0001$). One hundred and fifty-three (80.1%) of 191 children in the CQ group and 32 (16.6%) of 193 in the CQ + AS group received rescue treatment before day 14 or were parasitaemic on day 14 (OR = 20.2, 95% CI 11.7–35.4, $P < 0.0001$) (Table 2). By day 28, the number of children who were given rescue treatment or were parasitaemic had increased to 167/191 (87.4%) in the CQ group and to 85/194 (43.8%) in the CQ + AS group (OR = 8.9, 95% CI 5.2–15.4, $P < 0.001$). Genotyping from paired blood samples was carried out in 88% (110/125) of all recurrent infections. Thirty-four infections were classified as new and 53 as recrudescent while 23 remained undetermined. After excluding recrudescent and/or undetermined infections the parasitological failure rate at day 28 was estimated to be 77.4–81.1% in the CQ group and 24.2–32.4% in the CQ + AS group.

The proportion of clinical failures by day 14 was 67% (128/191) in the CQ group and 6.2% (12/193) in the CQ + AS group (OR = 30.6, 95% CI 15.3–62.7, $P < 0.001$). Clearance of parasites was significantly faster for children in the CQ + AS compared with the CQ group: (i) by day 1, 74.4% (145/195) vs. 96.9% (190/196) still parasitaemic, $P < 0.0001$; (ii) by day 2, 14.9% (29/194) vs. 88.4% (168/190), $P < 0.0001$; and (iii) by day 3, 3.1% (6/194) vs. 73.8% (121/164), $P < 0.0001$. Similarly, fever clearance was faster in the CQ + AS compared with the CQ group: (i) by day 1, 10.3% (20/195) vs. 36.7% (72/196) still with fever, $P < 0.001$; and (ii) by day 2, 2.6% (5/194) vs. 23.2% (44/190), $P < 0.001$. Paracetamol use was similar in the 2 groups of children (details not shown). Few children had gametocytes at recruitment (Table 1). Among children without gametocytes at recruitment, gametocyte carriage was significantly lower in the CQ + AS group at day 7 (CQ alone, 26.6% (34/128); CQ + AS, 5.5% (10/181); $P < 0.0001$) and day 14 (CQ alone, 10.8% (7/65); CQ + AS, 2.2% (4/178); $P < 0.01$). After day 14 the difference was not statistically significant although the percentage of gametocyte carriers was still higher in the CQ group. At enrolment the mean PCV was 31.2% in children treated with CQ alone and 32.2% in those who received CQ + AS. By day 7, the mean PCV was 30.5% in the CQ group and 32.5% in the CQ + AS group ($P = 0.03$) and the mean difference between days 0 and 7 was 0.16 and 1.25, respectively ($P = 0.02$). However, the PCV at day 14 and at day 28 was not significantly different between the 2 study groups (data not shown).

Discussion

Our study shows that the combination of CQ + AS was safe and well tolerated; there was no documented serious drug-related toxicity. Combination CQ + AS

Table 2. Causes of parasitological failure at days 14 and 28 in two study groups of children with acute uncomplicated *Plasmodium falciparum* malaria, Sao Tome and Principe, May–December 1999

	Chloroquine alone *n* (%)	Chloroquine + artesunate *n* (%)
Parasitological failure at day 14	($n = 191$)	($n = 193$)
Severe malaria signs	18 (9.4)	4 (2.1)
Adverse event requiring treatment withdrawal	1 (0.005)	0
Parasite density day 2 ≥ day 0	17 (8.9)	0
Parasite density day 3 ≥ 25% day 0 and patient unwell	22 (11.5)	0
Parasite density day 4 ≥ 25% day 0	2 (1.0)	0
Parasitaemia day 7	61 (31.9)	2 (1.0)
Recurrent parasitaemia at any time	32 (16.7)	26 (13.5)
Total	153 (80.1)	32 (16.6)
Parasitological failure at day 28	($n = 191$)	($n = 194$)
Recurrent parasitaemia at any time	14 (7.3)	53 (27.3)
Total	167 (87.4)	85 (43.8)
Parasitological failure at day 28 adjusted by genotyping	($n = 191$)	($n = 194$)
Only new infections excluded	155 (81.1)	63 (32.4)
New and untyped infections excluded	148 (77.4)	47 (24.2)

significantly improved treatment efficacy, symptom resolution and parasite clearance, and reduced gametocyte carriage. This is consistent with what was reported in The Gambia for SP + AS (von Seidlein *et al.*, 2000) and in Gabon, Senegal and Kenya for amodiaquine + AS (Adjuik *et al.*, 2002).

Both parasitological and clinical resistance rates to CQ were higher than previously anticipated in Sao Tome. The past decade has seen a progressive decrease of CQ efficacy. In 1990, among 58 children aged 5–11 years, RI resistance was 9% and RII resistance was 14% (Martet *et al.*, 1991). In 1997, CQ clinical failure was 20% in children aged < 11 years and parasitological failure rates in 1995–96 were estimated to be around 45% (Centro Nacional de Endemias, Sao Tome and Principe, documentos técnicos, 1995–1997). The results of an *in vitro* test carried out in 1994 revealed an extremely high CQ resistance, while there was no resistance to mefloquine and quinine (Lourerio *et al.*, 1996). Although CQ + AS significantly improved the outcome at days 14 and 28 compared with CQ alone, the parasitological failure rate by day 28 was still unacceptably high.

Only about one-third of the genotyped samples were identified as new infections. However, the technique used has its own limitations. For example, some genotypes detected during follow-up may not be detected at day 0 because they represent a minority of the parasite population and the infection would be wrongly classified as a new infection. Daily differences in the diversity of an infection have also been observed (Farnert, 2002) and, in places where there are few circulating parasite genotypes, new infections might have a similar genotype to the one eliminated by the drug (Greenwood, 2002). Even after excluding new infections, the day 28 parasitological failure rate of the combination was 25–30%. This clearly demonstrates that AS-containing combination therapies must be introduced before the sensitivity to the companion drug is compromised by resistance.

The results of this study have important implications for the antimalarial drug policy in Sao Tome and Principe and are being used to prepare new treatment guidelines. The benefits of an AS-containing combination in parasite and fever clearance, symptoms resolution, and in achieving a superior cure rate have been confirmed. However, the current levels of CQ resistance preclude the use of this combination in Sao Tomé where CQ should be abandoned as first-line drug. In other settings, where background rates of CQ resistance are lower, AS + CQ might be an option.

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