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Efficacy of chloroquine and sulfadoxine/pyrimethamine for the treatment of uncomplicated falciparum malaria in Koumantou, Mali

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Summary We report the results of an in vivo antimalarial efficacy study with chloroquine (CQ) and sulfadoxine/pyrimethamine (SP) conducted between 2003 and 2004 in Koumantou, southern Mali. A total of 244 children were included in the study; 210 children were followed-up for 28 days according to WHO recommendations, with PCR genotyping to distinguish late recrudescence from re-infection. Global failure proportions at Day 14, without taking into account re-infections, were 44.2% (95% CI 34.9—53.5%) in the CQ group and 2.0% (95% CI 0.0—4.8%) in the SP group. PCR-adjusted failure proportions at Day 28 were even higher in the CQ group (90.5% (95/105), 95% CI 84.8—96.2%) and relatively low in the SP group (7.0% (7/100), 95% CI 1.9—12.1%). These results show that CQ is no longer efficacious in Koumantou. The use of SP in monotherapy is likely to compromise its efficacy. We recommend the use of artemisinin-based combination therapy as first-line treatment for uncomplicated *Plasmodium falciparum* malaria in Koumantou.
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1. Introduction

In Africa, resistance of *Plasmodium falciparum* to common antimalarials represents a major obstacle for malaria control. Resistance to antimalarials is responsible for an increase in morbidity and mortality in many sub-Saharan countries (Snow et al., 2001). Evidence from western and southern Africa shows that recent improvements in childhood mortality due to diarrhoeal and vaccine-preventable diseases have been counterbalanced by rising malaria deaths (Korenromp et al., 2003). In Mali, malaria is a major health problem with an annual incidence of 40.9 per 1000, accounting for 33% of all consultations for health care (WHO Roll Back Malaria, 2001). Children under 5 years are the major at-risk population and account for 63% of total cases. In this age group, 59% of all deaths are due to malaria.

Resistance levels to chloroquine (CQ), the first-line recommended drug, of 17—30% have been reported (Plowe et al., 2001). A recent study reported 60% resistance to CQ in Faladje (Sangho et al., 2004). However, 14-day follow-up studies were used, which have been shown to have low sensitivity and no predictive value (Stepniewska et al., 2004). A study conducted a decade ago (Diourte et al., 1999) showed high efficacy of sulfadoxine/pyrimethamine (SP), the second-line drug in Mali, and a recent molecular-based study (Djimde et al., 2004) did not find the *DHFR-DHPS* quintuple mutant genotype associated with in vivo SP failure (Kublin et al., 2002). More current data in different malaria transmission areas of Mali are needed in order to update treatment policy-makers. Therefore, a study was carried out by Médecins sans Frontières, the Malian Ministry of Health and the Malaria Research and Training Centre (MRTC), University of Bamako, to evaluate the efficacy of these two antimalarial drugs.

2. Methods

2.1. Study site

The study was carried out between October 2003 and March 2004 in the town of Koumantou, in the Sikasso region located in the southern part of Mali. The Koumantou area is a rural area with 27 000 inhabitants spread over 23 villages. Malaria is present throughout the year, with a marked increase during the rainy season (i.e. May—November). The site was selected because of the existence of a functional health centre with well trained personnel as well as the presence of Médecins sans Frontières in the neighbouring town of Bougouni. A survey conducted in 2000 in this area (Thera et al., 2000) documented common use of CQ, often given at an inappropriate dosage.

2.2. Study design and procedures

The study adhered to the latest WHO guidelines for assessment of therapeutic efficacy of antimalarial treatment in high transmission settings (WHO, 2003). The study protocol was approved by the ethics committee of the Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali.

Children aged 6—59 months living within <20 km from the centre, weighing >5 kg and with suspicion of malaria (axillary temperature $\geq$37.5 °C) were screened. Children with a *P. falciparum* monoinfection and asexual parasitaemia between 2000/μl and 200 000/μl, no signs of severity or severe malaria (including severe anaemia defined by haemoglobin <5 g/dl), no history of allergic reactions to the respective study drugs, no presence of a concomitant febrile condition with the potential to confound the study outcome, and no severe malnutrition were included if their guardians consented in writing. Recent intake of antimalarials was not an exclusion criteria. As no information on the efficacy of these drugs in the study area was available for any of the drugs, sample size estimates were based on a 50% failure proportion for each drug. Applying a level of significance of 95% and a precision of 10%, 96 children per treatment arm were required. Allowing for a 10% loss to follow-up, the final sample size was thus raised to 106 children per treatment arm, i.e. 212 children in total.

Children received one of the study drugs under direct observation and were followed-up for 28 days. Because the transmission season was well advanced and to ensure that recruitment could be finished in at least one of the arms, the two drugs under study were tested consecutively, starting with CQ. Outcomes were both clinical and parasitological, with PCR genotyping performed to distinguish re-infections from recrudescences due to therapeutic failure.

2.3. Treatment and follow-up

Upon inclusion, children received either: CQ 25 mg/kg base given at 10 mg/kg on Day 0 and Day 1 and at 5 mg/kg on Day 2 (Nivaquine®; Rhône Poulenc, France); or SP, 1.25 mg/kg of pyrimethamine and 25 mg/kg of sulfadoxine on Day 0 (Fansidar®; Roche, Basel, Switzerland). Dosages were expressed as fraction of tablets and adapted to simplify prescription. Drugs were crushed and mixed with water and sugar, given in a spoon or a syringe to smaller children. All doses were directly observed and repeated if vomiting occurred within 30 min. Post-treatment visits were on Days 3, 7, 14, 21 and 28, or any other day in the case of recurrent illness. At each visit, children were assessed clinically, axillary temperature was recorded, and thick and thin blood films were inspected. Haemoglobin was measured on Days 0, 14 and 28. Patients who presented haemoglobin <7 g/dl received iron supplementation.

Rescue therapy in cases of uncomplicated malaria was oral artemisinin-based combination therapy (ACT) of artemether 20 mg and lumefantrine 120 mg (AT + LU; Coartem®; Novartis, Zurich, Switzerland), whilst i.v. or intrarectal quinine (10 mg/kg/8 h) followed by oral AT + LU was used in cases of complicated malaria.

On Day 0, a blood sample on filter paper was also taken for genotypic analysis and a second sample was collected in cases of symptomatic parasitaemia on or after Day 9, and at the end of follow-up in the presence of parasitaemia without symptoms.

2.4. Endpoint classification

Efficacy endpoints (failure or cure) were assigned according to the latest WHO classification (WHO, 2003). Patients were classified as early treatment failure (ETF) if they met any of the following criteria: (i) progression to severe malaria in the presence of parasitaemia on Days 1, 2 or 3; (ii) parasitaemia on Day 2 higher than on Day 0; (iii) any density of parasitaemia on Day 3 in the presence of fever; or (iv) parasitaemia on Day 3 $\geq$25% of the Day 0 count, irrespective of fever. After Day 3, patients with recurrent parasitaemia were classified as late clinical failure (LCF) if febrile, or late parasitological failure (LPF) if they remained afebrile until Day 28. All other children were classified as adequate clinical and parasitological response.

Children were withdrawn from the study in the case of: (i) vomiting any study dose twice; (ii) withdrawal of consent; (iii) onset of a serious febrile illness other than malaria; (iv) intake of any drug with antimalarial properties; (v) skipping any treatment dose; (vi) mixed parasitaemia; or (vii) any protocol violation. Patients who missed follow-up visits and did not come on successive days despite tracing were considered lost to follow-up.

2.5. Laboratory procedures

Capillary blood was obtained by fingerprick sampling. Microscopic examination was performed according to WHO guidelines (WHO, 1996). Thick and thin films were prepared on the same slide and stained with 10% Giemsa (pH 7.2) for 15 min. Asexual parasitaemia was quantified against 200—500 leukocytes, assuming a white blood cell count of 8000/μl. All the Day 0, Day 2 and Day 3 slides, and at least 10% of the follow-up slides, were re-read randomly every day and any discordance was resolved by a third reader. External quality control on a random sample of slides (n = 152) was carried out at the MRTC in Bamako, and by an independent laboratory technician in France, yielding an endpoint affecting discordance in <4% of cases. Haemoglobin was measured using a HemoCue® type haemoglobinometer (HemoCue AB, Ängelholm, Sweden) (von Schenck et al., 1986).

In high-transmission settings, re-infections occur frequently and confound LCF and LPF endpoints. To identify true failures (recrudescence), blood samples were collected on Whatman No. 3 filter paper and analysed at the MRTC by PCR of *P. falciparum* DNA extracted from the Day 0 and failure day samples. Procedures in this laboratory relied on well described protocols (Djimde et al., 2001; Ranford-Cartwright et al., 1997) comparing the Day 0 and failure day alleles of the merozoite surface proteins msp-1 and msp-2 as well as *glurp* gene loci. Possible outcomes were: (i) recrudescence if the alleles of the pre- and post-treatment samples were the same for msp-1, msp-2 and *glurp*; (ii) re-infection if the alleles of the pre- and post-treatment samples were distinct; (iii) mixed recrudescence and re-infection if similar alleles were found in the pre- and post-treatment samples for all the markers as mentioned above, but with additional distinct alleles identified; or (iv) no DNA isolated, if either or both the pre- and post-treatment samples could not be amplified.

2.6. Data entry and analysis

Data were recorded in an individual record form and entered into a locked Excel® spreadsheet and all entries were verified. The data set was analysed using Stata® 8.02 (Stata Corp., College Station, TX, USA). Baseline characteristics of patients in each group were compared using a χ^2 test for categorical variables and the Mann—Whitney *U*-test for continuous variables non-normally distributed. Failure proportions at Day 14 and Day 28 were calculated as the total number of failures (ETF + LCF + LPF) over the total number of analysable outcomes, and expressed as a percentage with associated 95% CI. PCR analysis results were used to adjust failure proportions at Days 9—28. Only treatment failures proven to be recrudescence or mixed recrudescence and re-infection were classified as true failures. Patients for which the PCR result was inconclusive (no DNA isolated) or reported as a pure re-infection, patients lost to follow-up and secondary exclusions were all excluded from the analysis.

3. Results

A total of 382 children were screened, of which 244 met the inclusion criteria: 142 were assigned to the CQ arm and 102 to the SP arm (Figure 1). One patient (0.4%) was lost to follow-up and 33 (13.5%) were withdrawn (31 in the CQ arm and 2 in the SP arm). Reasons for withdrawal included intake of antimalarials (4), mixed parasitaemia (4), onset of serious illness (2), allergy to study drug (1), twice vomiting the study drug (11) and protocol violation (11). Amongst the protocol violations, eight were due to parasitaemia >200 000/μl and three to parasitaemia <2000/μl that appeared at the second reading of the Day 0 slides. The number of patients analysable at Day 14 was 113 and 100 in the CQ and SP arms, respectively. The number of patients analysable at Day 28 was 110 and 100 in the CQ and SP groups, respectively. Baseline characteristics were similar across treatment groups, with the exception of temperature, haemoglobinaemia and parasite density: children had a higher temperature (P = 0.04), were more anaemic (P = 0.01), but had a lower parasitaemia (P < 0.01) in the CQ group than in the SP group (Table 1).

Global failure proportions at Day 14, without taking into account re-infections, were 44.2% (95% CI 34.9—53.5%) in the CQ group and 2.0% (95% CI 0.0—4.8%) in the SP group. PCR-adjusted failure proportions at Day 28 were even higher in the CQ group (90.5% (95/105), 95% CI 84.8—96.2%), but were still low in the SP group (7.0% (7/100), 95% CI 1.9—12.1%); ETFs were frequent in the CQ group (29.5% (31/105), 95% CI 20.7—38.4%); in the SP group, there were two ETFs (Table 2). Only 16.0% (17/106) of CQ-treated patients but 83.0% (83/100) of those receiving SP were parasite free on Day 3. In the CQ group, the majority of treatment failures were pure or mixed recrudescence (90.5% (57/63)), whereas none of the children in the SP arm experienced a re-infection.

4. Discussion

This study provides estimates of the in vivo antimalarial efficacy of first- and second-line drugs in Mali, where current

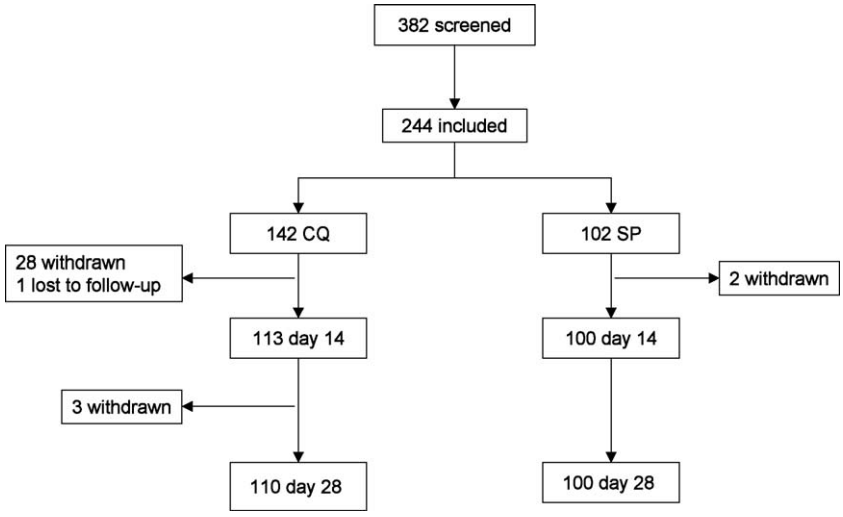

Figure 1 Details of study inclusion and follow-up of patients in the in vivo antimalarial efficacy study, Koumantou, Mali, 2003—2004. CQ: chloroquine; SP: sulfadoxine/pyrimethamine.

Table 1 Baseline (Day 0) characteristics of patients included in the in vivo antimalarial efficacy study, Koumantou, Mali, 2003—2004

Characteristic	CQ ($n = 142$)	SP ($n = 102$)	*P*-value
Age (years) (median (IQR))	2 (1—3)	2 (1—3)	0.24
Sex (ratio M:F)	1.0	1.5	0.16
Antimalarial treatment before inclusion, *n* (%)	115 (81.0)	89 (87.3)	0.19
Axillary temperature (°C) (median (IQR))	38.6 (38.1—39.2)	38.4 (37.9—39.1)	0.04
Haemoglobin (g/dl) (median (IQR))[a]	7.7 (6.5—9.2)	8.3 (7.2—9.7)	0.01
Moderate anaemia (<8 g/dl), *n* (%)	77 (55.0)	43 (42.2)	
Mild anaemia (8—10 g/dl), *n* (%)	59 (42.1)	51 (50.0)	
No anaemia (≥11 g/dl), *n* (%)	4 (2.9)	8 (7.8)	
Asexual parasitaemia (μl) (geometric mean (IQR))[b]	31 496 (11 580—84 920)	56 993 (27 600—132 000)	<0.01

CQ: chloroquine; SP: sulfadoxine/pyrimethamine; IQR: interquartile range (25—75%).
[a] Two missing values.
[b] Three missing values.

data regarding CQ and SP were urgently needed. Methodologically, the 28-day follow-up combined with adjustment of the results by PCR analysis provided more realistic estimates of efficacy than the traditional 14-day studies.

The major limitation of our study was the method of treatment allocation, which was not randomised. This could explain the unequal level of fever, haemoglobin and parasite density at inclusion and does not allow a strict comparison of results between the two arms. A second limitation is the relatively high number of withdrawals in the CQ arm owing to some misunderstandings by the staff at the beginning of the study. Nevertheless, measures taken (such as further training of nurses and laboratory staff) have probably limited potential biases. The validity of our data is further supported by the almost non-existent loss to follow-up and the low proportion of slide discordance.

Table 2 Failure proportions at Day 28 (clinical and parasitological failures, after PCR adjustment) for the in vivo antimalarial efficacy study, Koumantou, Mali, 2003—2004

Outcome	CQ ($n = 105$)		SP ($n = 100$)	
	n (%)	95% CI	*n* (%)	95% CI
ETF	31 (29.5)	20.7—38.4	2 (2.0)	0.0—4.8
LCF	41 (39.0)	29.6—48.5	0 (0.0)	—
LPF	23 (21.9)	13.9—29.9	5 (5.0)	0.7—9.3
Global failure (ETF + LCF + LPF)	95 (90.5)	84.8—96.2	7 (7.0)	1.9—12.1

CQ: chloroquine; SP: sulfadoxine/pyrimethamine; ETF: early treatment failure; LCF: late clinical failure; LPF: late parasitological failure.

We have shown that the in vivo resistance to CQ, the first-line antimalarial treatment in Mali, was unquestionably very high in Koumatou area, confirming previous reports from MRTC surveys (Sangho et al., 2004) and from surveys conducted in east and west Africa (Abacassamo et al., 2004; Checchi et al., 2005; Stivanello et al., 2004). CQ resistance is thus well above the 25% value considered by the WHO as the threshold for changing the drug policy (WHO, 2003). The dramatic impact of CQ resistance on childhood morbidity and mortality has been well described in western Africa (Trape, 2001). In Kenya, 69% of deaths due to malaria were attributed to ineffective CQ treatment (Zucker et al., 2003).

In vivo resistance to SP, which was the second-line antimalarial in Mali, was relatively low, well below the WHO threshold and confirming the existing MRTC data (Coulibaly et al., 2002; Diourte et al., 1999). However, SP should not be used as monotherapy as resistance to SP is known to develop rapidly (White, 1992). Furthermore, recent studies in Sudan (Stivanello et al., 2004) and Sierra Leone (Checchi et al., 2005) reported unacceptable levels of resistance to this drug.

In the face of spreading antimalarial drug resistance, the WHO recommends that monotherapeutic options be abandoned in favour of combination therapy (WHO, 2001). This approach, already in use for tuberculosis and AIDS, is essential not only to improve efficacy but also to protect remaining viable antimalarials against further spread of resistant strains (Nosten and Brasseur, 2002). Currently, the WHO recommends that combinations including an artemisinin derivative be prioritised for deployment (WHO, 2002).

The results of this study clearly discourage the combination of CQ with artemisinin derivatives. SP + artesunate (AS) has been tested elsewhere in Africa and showed good efficacy in sites where resistance to SP was low (Abacassamo et al., 2004; von Seidlein et al., 2000), but proved relatively disappointing where SP resistance was already well developed (Olliaro and Taylor, 2003; Priotto et al., 2003). This ACT represents a good alternative to be used as first-line antimalarial treatment in Koumantou. AT + LU is another ACT that could be proposed. It is a highly efficacious fixed combination but is relatively expensive (approximately US$2.4 for treating an adult). Amodiaquine (AQ) + AS is another option, providing that AQ efficacy remains acceptable. Its price, when available in blister, is similar to that of the SP + AS combination (approximately US$1.5), and a fixed combination should be marketed in the near future.

Our data support the decision made by the Ministry of Health of Mali, which recently changed its malaria treatment guidelines and adopted AS + AQ or AT + LU as first-line therapy for uncomplicated malaria.

Conflicts of interest statement

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

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