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Efficacy of chloroquine, sulphadoxine–pyrimethamine and amodiaquine for treatment of uncomplicated *Plasmodium falciparum* malaria in Kajo Keji county, Sudan

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Summary

To provide advice on the rational use of antimalarial drugs, Médecins Sans Frontières conducted a randomized, an open label efficacy study in Kajo Keji, an area of high transmission of malaria in southern Sudan. The efficacy of chloroquine (CQ), sulphadoxine–pyrimethamine (SP) and amodiaquine (AQ) were measured in a 28-day *in vivo* study, with results corrected by PCR genotyping. Of 2010 children screened, 115 children aged 6–59 months with uncomplicated *Plasmodium falciparum* malaria were randomized into each group to receive a supervised course of treatment. Of these, 114, 103 and 111 were analysed in the CQ, SP and AQ groups, respectively. The overall parasitological failure rates at day 28 were 93.9% [95% confidence interval (CI) 87.3–97.3] for CQ, 69.9% (95% CI 60.0–78.3) for SP, and 25.2% (95% CI 17.7–34.5) for AQ. These results provide important missing data on antimalarial drug efficacy in southern Sudan. They indicate that none of the drugs could be used in monotherapy and suggest that even in combination with artemisinin, cure rates might not be efficacious enough. We recommend a combination of artemether and lumefantrine as first-line treatment for uncomplicated *P. falciparum* malaria cases in Kajo Keji county.

keywords malaria, *Plasmodium falciparum*, resistance, sulphadoxine–pyrimethamine, amodiaquine, chloroquine, Sudan

Introduction

Apart from a 10-year period of relative peace from 1972 to 1983, Sudan has been divided by civil war for nearly half a century. The long-lasting war left the southern part of the country in administrative, economic, political and social decay. In this state of complex emergency, malaria, the leading health problem in Sudan, is inadequately controlled (WHO 2000). It is estimated that in the southern sector of Sudan, 24–36% of the population are affected with malaria (UN/OCHA 2002).

In Kajo Keji County, in the south of Sudan where Médecins Sans Frontières (MSF) supports Mundari Hospital, malaria accounts for 32% of the total consultations, 51% of the under five children consultations and 26% of hospital deaths (MSF, unpublished data). The first-line treatment of uncomplicated *Plasmodium falciparum* malaria was chloroquine (CQ) with sulphadoxine–pyrimethamine (SP) as second and quinine as third-line treatment. Verbal accounts from the local clinicians suggested that CQ and SP were losing their efficacy, however no recent information concerning resistance to these drugs was available. Moreover, there are only limited data on the current efficacy of antimalarial drugs in other parts of south Sudan (van den Broek *et al.* 2003). To fill this data gap and explore possible alternatives to the first-line treatment used in this site, we decided to conduct an *in vivo* efficacy study of CQ, SP, and amodiaquine (AQ) among children under the age of 5 in Kajo Keji.

Methods

Study site and duration

The study was conducted in a separate clinic of Mundari Hospital, where MSF has been working since 1997. It is the referral hospital of Kajo Keji County (150 000 inhabitants), situated in western Equatoria near the border with

Uganda. Malaria transmission is perennial with peaks during the rainy seasons of May–August and January/February. Ethical approval for the study was obtained from the Sudan Relief and Rehabilitation Association (SRRA) authorities.

Patients, study procedures and treatment

The study protocol was based on the 1996 WHO guidelines (WHO 1996). Patients with fever (axillary temperature ≥37.5 °C) or history of fever in the past 24 h were screened at the hospital outpatients department and referred to the laboratory and then to the study clinic. Children aged 6–59 months with *P. falciparum* mono-infection and asexual parasitaemia of 1000–100 000/μl were eligible for the study. They were excluded if at least one of the following criteria was present: (i) signs of severe malaria (WHO 1996); (ii) severe malnutrition (weight-for-height <70% of the median or bilateral oedema); (iii) severe infectious disease; (iv) history of hypersensitivity reactions to the drugs under evaluation; (v) intake of a complete treatment of CQ, SP or AQ within the previous 3 days; or (vi) residence at more of 10 km from the hospital. Written, informed consent was obtained by signature or fingerprint from parents or guardians. Patients were randomly allocated to one of the three treatment groups: (i) CQ base tablet of 150 mg (Avloclor®, ZENECA, at a dose of 25 mg/kg base divided over 3 days); (ii) sulphadoxine 500 mg + pyrimethamine 25 mg (Fansidar®, Roche, as a single dose of 1.25 mg/kg); (iii) amodiaquine hydrochloride base tablet of 200 mg (Camoquine, Parke-Davis, France, at a dose of 30 mg/kg base divided over 3 days). Allocation followed a block randomization procedure (blocks of 10). Treatments were kept in sealed envelopes that were opened at the end of the inclusion procedure, when the child received the treatment. All doses were directly observed by the study clinician; the complete dose was repeated in case of vomiting within 30 min, while half the dose was repeated in case of vomiting after 30–60 min.

Follow-up visits with clinical and parasitological examinations were scheduled on days 1, 2, 3, 7, 14, 21 and 28. Haemoglobin was measured at day 0. Samples for PCR genotyping were taken at entry and in case of treatment failure after day 10 of follow-up. Rescue therapy for patients failing treatment with AQ or CQ was SP, while patients failing treatment on SP were treated with quinine.

Children were secondarily withdrawn from the study in case of: (i) allergic reaction to the study drug; (ii) movement of the patient outside the study site; (iii) withdrawal of consent; (iv) intake of drugs with antimalarial activity; (v) detection of mixed malaria infection during days 2 or 3; (vi) vomiting of any of the antimalarial drug more than twice; (vii) occurrence of a concomitant disease that would interfere with the classification of the outcome. Patients who missed follow-up visits and did not come on successive days despite tracing were considered lost to follow-up.

Therapeutic outcomes

Therapeutic outcomes were determined according to parasitological and clinical observations made during follow-up. Early treatment failure (ETF) was defined in case of: (i) severe malaria on days 1, 2 or 3 in the presence of parasitaemia, or (ii) axillary temperature ≥37.5 °C on day 2 with parasitaemia greater than that of day 0, or (iii) axillary temperature ≥37.5 °C on day 3 with parasitaemia, or (iv) parasitaemia on day 3 > 25% of that of day 0. Late treatment failure (LTF) was defined in case of: (i) development of danger signs or signs of severe malaria after day 3 in the presence of parasitaemia without previously meeting any of the criteria of ETF, or (ii) presence of parasitaemia on any day after day 3 without previously meeting any of the criteria of ETF. Absence of ETF and LTF defined an adequate treatment response (ATR). After PCR genotyping, reinfections were reclassified as ATR and recrudescences were reclassified as LTF. When the PCR was non-available, the case was excluded from the analysis.

Laboratory methods

Capillary blood was obtained by fingerprick. Thick blood films were stained with 10% Giemsa for 20 min. Parasite density was quantified against 200 leucocytes on a thick film, assuming a total leukocyte count of 8000 per μl (WHO, 1991). All slides were read by the first laboratory technician and then cross-checked by a laboratory supervisor. A random sample of slides was sent to the African Medical and Research Foundation (AMREF) for an external quality control. Haemoglobin levels were measured using the Lovibond undiluted technique (Assistant Co., Sondheim Rhön, Germany). Blood samples for PCR genotypic analysis were collected on Whatman N °3 filter paper and analysed at the Kenya Medical Research Institute according to a published method (Ranford-Cartwright *et al.* 1997). The three *P. falciparum* antigenic gene loci of merozoite surface protein-1 (msp-1), merozoite surface protein-2 (msp-2), and glutamate-rich protein (GLURP) were used in differentiating recrudescence from reinfection. The band profiles of paired pre-treatment and failure-day samples were compared. Paired samples with different band profiles were classified as reinfections,

whereas a recrudescence was concluded if the band profiles were similar. If the profiles were similar but there were additional or missing bands in either sample, a recrudescence was also concluded.

Data management and analysis

The sample size was calculated using a prevalence of resistance of 50% for each drug. With a precision of 10% and a type 1 error of 0.05, the sample size was 96 persons for each drug. Allowing for a loss to follow-up of 20%, the final sample size was of 115 children in each treatment group. Data were entered in a Microsoft Excel 2000 database. Each record was individually checked. Data were analysed using SPSS 10.0.5 (SPSS Inc., Chicago, IL, USA) and Epi-Info 6 (CDC, Atlanta, GA, USA). Failures were expressed as proportions and corresponding 95% confidence intervals were calculated. Comparisons between proportions were made using the χ^2 test. Two-tailed *P*-values <0.05 were considered significant. Failures classified as recrudescence after PCR analysis were confirmed as treatment failures, whereas when the PCR result showed a reinfection, the patient was reclassified as an ATR.

Results

From 26 October 2001 to 28 January 2002, of 2010 children referred to the study clinic 1665 were excluded (Figure 1). Main reasons for exclusions were (many were excluded for more than one reason): negative malaria smear ($n = 944$), age not corresponding ($n = 688$), mixed parasitaemia ($n = 307$), parasitaemia below 1000/μl

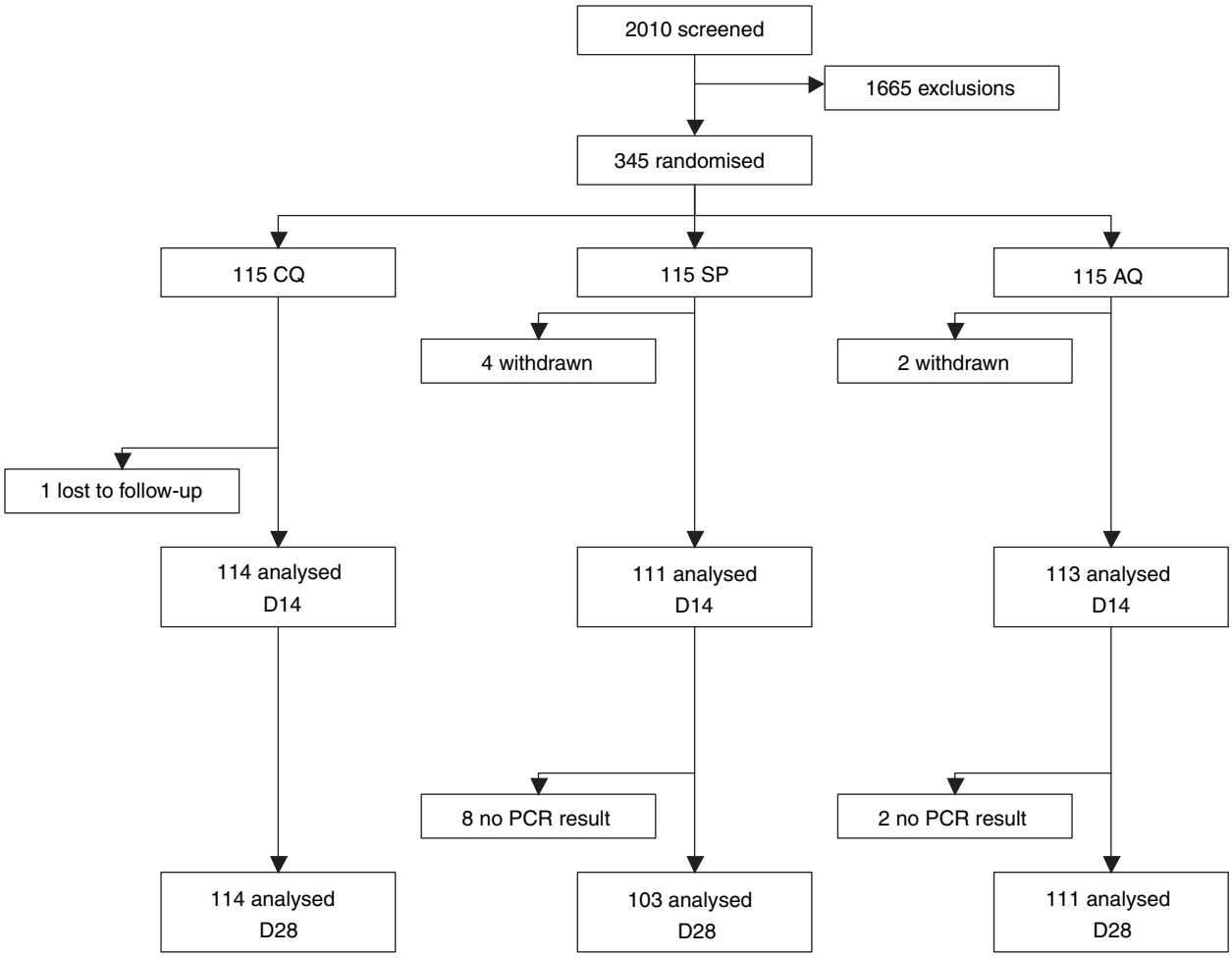

Figure 1 Details of study inclusions and follow-up.

($n = 261$) or above 100 000/μl ($n = 1$). A total of 345 patients were randomized (115 in each study group), with no statistically significant differences in baseline characteristics between the three groups (Table 1). After inclusion, one patient was lost to follow-up (in the CQ group), and six patients were subsequently excluded (four in the SP group and two in the AQ group). Ten samples failed to generate PCR results (eight in the SP group and two in the AQ group) and were therefore excluded from the day 28 analysis.

Table 2 shows the therapeutic outcomes at days 14 and 28, before and after PCR adjustment. The vast majority of late failures were asymptomatic: 74/77 (96%) in the CQ group, 87/94 (92%) in the SP group and 86/92 (93%) in the AQ group. At day 14, the proportion of failures was 89.5% in the CQ group, 54.0% in the SP group and 21.2% in the AQ group. After PCR adjustment, the proportion of failures at day 28 were 93.9% for CQ, 69.9% for SP and 25.2% for AQ. The proportion of failures was significantly higher in the CQ group than in the SP and the AQ groups ($P < 0.01$ for each comparison) and significantly higher in the SP group than the AQ group ($P < 0.01$). The time of occurrence of failures seemed correlated with the level of drug resistance: before day 14 in 91.5% (98/107) of failures in the CQ group, in 68.0% (49/72) in the SP group and in 53.6% (15/28) in the AQ group. The external quality control performed on 94 slides showed 97.8% concordance.

Discussion

This study provides important missing data on the efficacy of CQ, SP and AQ for treatment of *P. falciparum* malaria in southern Sudan, where data on antimalarial efficacy are scarce. The high failure rates to CQ (93.9%) and SP (69.9%) are alarming, and confirm the clinical observations of the local clinicians. The low efficacy of CQ can be explained by the frequent use of the drug within and

Table 1 Baseline characteristics of included patients

Characteristic	CQ ($n = 115$)	SP ($n = 115$)	AQ ($n = 115$)
Age (months), mean (SD)	26.1 (16.4)	23.6 (15.2)	23.6 (15.1)
Gender ratio (male/female)	0.80 (51/64)	1.16 (62/53)	1.35 (66/49)
Axillary temperature (°C), mean (SD)	37.4 (0.9)	37.5 (1.0)	37.4 (1.0)
Haemoglobin (g/dl)	10.5	10.4	10.1
Mean (SD)	(1.5)	(1.5)	(1.5)
% <11 g/dl	59.1	66.1	68.7
Parasite density (/μl), geometric mean (range)	10 100 (1040–98 256)	8850 (1000–96108)	11 765 (1000–98 799)

Table 2 Therapeutic outcomes with and without PCR adjustment

	No.	%	95% CI	No.	%	95% CI	No.	%	95% CI
	CQ ($n = 114$)			SP ($n = 111$)			AQ ($n = 113$)		
Before PCR adjustment									
Treatment failure at D14	102	89.5	83.8–95.1	60	54.0	44.7–63.2	24	21.2	14.3–30.1
Treatment failure at D28	114	100	95.9–100	100	90.1	82.6–94.7	92	81.4	72.7–87.9
ETF	37	32.5	22.2–42	6	5.4	2.2–11.9	0	0	0–4.1
LTF D4–D14	65	57	47.4–66.1	54	48.6	39.1–58.3	24	21.2	14.3–30.1
LTF D15–D28	12	10.4	5.8–18	40	36	27.3–45.8	68	60.2	50.5–69.1
Adequate response	0	0	0–4.1	11	9.9	5.3–17.4	21	18.6	12.1–27.2
				($n = 103$)			($n = 111$)		
After PCR adjustment									
Treatment failure at D14	98	85.9	79.5–92.3	49	47.5	37.9–57.2	15	13.5	8.0–21.5
Treatment failure at D28	107	93.9	87.3–97.3	72	69.9	60.0–78.3	28	25.2	17.7–34.5
ETF	37	32.5	24.2–41.9	6	5.8	2.4–12.8	0	0	0–4.1
LTF D4–D14	61	53.6	43.9–62.8	43	41.7	32.2–51.9	15	13.5	8–21.5
LTF D15–D28	9	7.9	3.9–14.9	23	22.3	14.9–31.8	13	11.7	6.6–19.5
Adequate response	7	6.1	2.7–12.7	31	30.1	21.6–40.0	83	74.8	65.5–82.3

outside the official health system where self-medication was frequently observed, a factor that favours the propagation of drug resistance (Wernsdorfer 1994). The low efficacy of the less prescribed SP corroborates the information showing that resistance to SP is increasing in Africa (White *et al.* 1999). The moderate failure rates to the not previously used AQ can perhaps be attributed to cross-resistance with CQ (Olliaro *et al.* 1996; Ochong *et al.* 2003), or perhaps to its inadequate use outside the official health system. The difference in the proportion of reinfections between the three groups (AQ: 56%; SP: 19%; CQ: 6%) may be explained by the level of drug resistance to each of these drugs. Moreover, the much higher proportion of reinfections in the AQ group compared with the SP group could be explained by the prophylactic effect of SP whose terminal elimination half-life is longer (3–5 weeks) than that of AQ (1–3 weeks) (Krishna & White 1996).

This information was collected through a 28-day randomized study with PCR adjustment. This design allowed the identification of late failures occurring after several weeks of follow-up, the adequate comparison between the two study groups and the distinction of recrudescences from reinfections (White 2002). The definition of failure was based on parasitological criteria only. This prevented us from distinguishing between parasitological and clinical failures, which is currently recommended by the WHO. Moreover, by giving rescue treatment to these asymptomatic parasitaemic patients, we were not able to identify patients who could eventually have cleared their parasitaemia spontaneously during follow-up. In this sense, we think that our results may over-estimate to some extent the level of drug failure. They also need to be compared with some caution to other studies performed in other areas of Sudan (National Malaria Administration/Unicef 1999; Epicentre 2003; van den Broek *et al.* 2003), considering the particular outcome definition used in Kajo Keji.

The WHO recommends 25% as a threshold of therapeutic failure above which a change in therapy should be implemented and adds that the process of looking for alternative therapy should start even earlier when failure rates reach 15–24% (Bloland *et al.* 1996; WHO 2001). Even if our study measured parasitological failure, and not clinical failure, on which the recommendations of WHO are based, the findings of this study indicate that a change in first-line treatment is necessary. A long-term approach is to consider combination therapy with an artemisinin derivative. This is now being promoted as the best option for treating *P. falciparum* malaria and retarding the emergence of resistance (White & Olliaro 1996; WHO/Roll Back Malaria 2003). The very high failure rates to CQ and SP observed in Kajo Keji preclude them as partners in such combination. The combination of artesunate with AQ would certainly increase the antimalarial efficacy compared with AQ monotherapy, but with the available information it is unclear whether acceptable cure rates could be reached in Kajo Keji. Moreover, given the failure rates of AQ found in our study, the useful therapeutic life of this combination could be short.

Another option is the combination of artemether and lumefantrine (Coartem®). Following this study, Coartem® replaced CQ as first-line treatment in Kajo Keji, after discussions between MSF and the health authorities. Most studies have shown that the six-dose regimen of this drug is highly effective and well tolerated (von Seidlein *et al.* 1997; van Vugt *et al.* 1998; Kshirsagar *et al.* 2000; Omari *et al.* 2003). However, this fixed combination is expensive (US$2.4/adult treatment), and adherence is likely to be low, considering the need of taking the drug together with a fatty meal. Monitoring and improving patient adherence are integral to the introduction of Coartem®, and showed several months after introduction of the drug in Kajo Keji that patient adherence was rather low (Depoortere *et al.* 2004). If we want to ensure treatment efficacy in the years to come, we need to ensure compliance with the regimen.

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