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Antimalarial efficacy of sulfadoxine–pyrimethamine, amodiaquine and a combination of chloroquine plus sulfadoxine–pyrimethamine in Bundi Bugyo, western Uganda

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Summary

We report below an *in vivo* antimalarial efficacy study conducted in 2002 in Bundi Bugyo, a district of western Uganda housing a large displaced population. We tested sulfadoxine–pyrimethamine (SP), amodiaquine (AQ) and the combination chloroquine plus SP (CQ + SP). A total of 268 children with uncomplicated *Plasmodium falciparum* malaria were followed-up for 28 days according to WHO recommendations, with PCR genotyping to distinguish late recrudescences from re-infections. PCR-adjusted failure proportions at day 28 were 37.0% (34/92, 95% CI 27.1–47.7) in the SP group, 20.6% (14/68, 95% CI 11.7–32.1) in the AQ group and 22.8% (18/79, 95% CI 14.1–33.6) in the CQ + SP group. Early failures were particularly frequent in the SP group (15.2%). Clearance of gametocytes was slower in the SP and CQ + SP groups than in the AQ group. This study suggests that, in Bundi Bugyo, CQ + SP (Uganda's first-line regimen) will need to be replaced by a more efficacious regimen. Across Uganda, the deployment of SP containing combinations may not be a feasible long-term strategy. For Bundi Bugyo, we recommend a combination of artesunate and AQ. Our study also confirms previous findings that resistance is considerably underestimated by 14-day follow-ups. Antimalarial policy decisions should therefore be based on 28-day studies, with PCR adjustment to distinguish re-infections.

keywords malaria, *Plasmodium falciparum*, efficacy, sulfadoxine–pyrimethamine, amodiaquine, chloroquine, Uganda

Introduction

Throughout Africa, increasing *Plasmodium falciparum* resistance to common antimalarials poses a formidable obstacle for malaria control. Uganda, which in 2001 declared 5 622 934 cases of malaria (39% of all outpatient consultations), is particularly affected by this problem (Ministry of Health of Uganda 2003). Therapeutic failure to chloroquine (CQ) reaches 76% in Kampala (Kamya *et al*. 2001), 81% in Mbarara (Legros *et al*. 2002) and 10–48% in other sentinel sites of the East African Network for Monitoring Antimalarial Treatment (EANMAT) (Kamya *et al*. 2002). Resistance to sulfadoxine–pyrimethamine (SP) is also increasing, with 18% failure in Kampala (Dorsey *et al*. 2002), 25% in Mbarara (Legros *et al*. 2002) and 6–19% in other EANMAT sites (Kamya *et al*. 2002). The above data are based on 14-day patient follow-ups, and clinical classification criteria (EANMAT 1999).

Bundi Bugyo District of western Uganda, bordering the Democratic Republic of the Congo, houses a large population displaced from mountain villages due to armed conflict. Since 1997, the medical relief organization Médecins Sans Frontières (MSF) has been supporting the district's health facilities. Malaria, diagnosed mostly on clinical grounds, seemed a dominant problem in Bundi Bugyo hospital, accounting from November 2000 to June 2001 for 33% of consultations, 49% of admissions and 50% of inpatient deaths (MSF, unpublished data). Little was known, however, about the efficacy of antimalarials in this region, apart from a 1997 study showing 33% and 5% clinical failure of CQ and SP, respectively (Kilian AHD, Prislin I, Kabagambe G *et al*., 1997, unpublished report).

In an effort to explore possible alternatives to CQ, we decided in 2001 to conduct an *in vivo* study of SP and amodiaquine (AQ) among children less than 5 years of age

in Bundi Bugyo. While the study was being implemented, Uganda introduced a new national recommendation for treatment of uncomplicated malaria, consisting of a combination of CQ and SP (CQ + SP) as first-line treatment. This provisional policy assumed that such a combination would provide better efficacy than CQ or SP alone, and protect partner drugs against further development of resistance; it equally reflected insufficient evidence on other potential alternatives (Kamya *et al*. 2002). CQ + SP, however, had not yet been studied in Uganda, and in Africa only one trial was reported (Bojang *et al*. 1998). In order to gain more information about the efficacy of this new protocol, we added a third, CQ + SP, group to our study.

Methods

Study site and population

The plains of Bundi Bugyo district (altitude 700–900 m) experience perennial malaria transmission, with peaks in July and December. *Plasmodium falciparum* is the predominant species reported (98%), with *Anopheles gambiae* and *Anopheles funestus* as the main vectors (Jelinek *et al*. 1996). At the time of writing, about 137 000 displaced persons were settled in 63 makeshift camps scattered across this area. An evaluation of a mass distribution of bednets in 2001 showed 76% coverage and a 9% community prevalence of *P. falciparum* infection (Spencer S., Grant A. D., Piola P. *et al*., submitted). We recruited children with uncomplicated *P. falciparum* malaria from the outpatient departments of Bundi Bugyo District Hospital, and the nearby Nyahuka Health Centre. A study clinic was established within Bundi Bugyo hospital. The study was approved and supported by the Ugandan Ministry of Health.

Inclusion criteria and procedures

The study was based on current WHO and EANMAT recommendations (EANMAT 1999; WHO 2002). Accordingly, children aged 6–59 months with fever (axillary temperature ≥37.5 °C) or history of fever in the past 24 h were screened from outpatient lines. In Nyahuka, a preliminary Paracheck®rapid test was also performed so as to minimize unnecessary transport to the study clinic. Children with a *P. falciparum* mono-infection and asexual parasitaemia 1000–100 000 μl^{-1} were eligible for the study. Exclusion criteria were (i) signs of severity or severe malaria (WHO 2000a), (ii) reported intake of a full course of antimalarials in the previous 7 days, (iii) history of allergic reactions to the study drug and (iv) presence of a concomitant febrile condition with the potential to confound study outcome (e.g. ARI, measles, severe diarrhoea, severe wounds, etc.; mild influenza cases were not excluded). Written, informed consent was obtained by signature or fingerprint from parents or guardians.

Treatment and follow-up

Study regimens consisted of SP 1.25 mg/kg stat (Falcidin®,Cosmos Ltd., Nairobi), AQ 30 mg/kg base divided into three daily doses of 10 mg/kg (Malaratab®, Cosmos Ltd., Nairobi) and CQ 25 mg/kg base given at 10 mg/kg on day 0 and day 1, and 5 mg/kg on day 2 (Ipca Laboratories Ltd., Mumbai), plus an SP stat dose as above. Study drugs came from approved EANMAT sources. All doses were directly observed and repeated after 30 min in case of vomiting or spitting. On day 0, haemoglobin and gametocyte carriage were also recorded, and a blood sample collected for possible genotypic analysis to distinguish recrudescences from re-infections.

After treatment (days 0, 1 and 2), children were re-assessed clinically and parasitologically on days 3, 7, 14, 21 and 28. If children were parasitaemic but asymptomatic during follow-up, additional home visits were performed so as to prevent aggravation (according to WHO and EANMAT guidelines, treatment is withheld for such children until either appearance of symptoms or day 28). Haemoglobin and gametocytaemia were re-measured on days 14 and 28, and a second blood sample collected in case parasites reappeared after day 9. Rescue therapy (quinine hydrochloride 10 mg/kg/8 h for 7 days) was administered upon treatment failure (see below).

The three regimens were tested sequentially (SP, then AQ, then CQ + SP). We decided against a randomized design mainly because of poor security in the area, leading to a strong risk of early study interruption, and thus failure to reach a sufficient sample size in any arm.

Outcome classification

Children were withdrawn from the study in case of (i) vomiting any study dose twice, (ii) serious allergic reaction to the study drug, (iii) onset of a serious febrile illness or (iv) intake of any drug with antimalarial properties. Children who skipped any dose or scheduled visit despite tracing were considered lost to follow-up. Remaining outcomes were classified according to WHO 2001 criteria: early treatment failure (ETF) in case of (i) progression to severe malaria by day 3, (ii) parasitaemia on day 2 > day 0, (iii) parasitaemia on day 3 ≥ 25% of day 0 or (iv) fever and parasitaemia on day 3; late clinical failure (LCF) any time from day 4 to day 28 in case of (i) progression to severe malaria or (ii) fever plus parasitaemia; and late parasitological failure (LPF) in case of afebrile parasitaemia

on day 28. All other children were considered to have had an adequate clinical and parasitological response (ACPR).

Laboratory methods

Capillary blood was obtained by finger prick. Thick and thin films, prepared on the same slide, were stained with 10% Giemsa (pH 7.2) for 12 min. Asexual parasitaemia was quantified against 200–500 leukocytes, assuming a normal level of 8000 leukocytes/μl (WHO 1991). Presence of gametocytes was noted. All study slides were re-read blindly by a second microscopist, and any discordances resolved by a third, experienced reader. External controls were conducted by a Ministry of Health expert, and at the Mbarara University of Science and Technology, where, on a sample of 97 slides, concordance was 91%. Haemoglobin was measured using the Lovibond technique (Assistant Co., Sondheim Rhon, Germany). Axillary temperature readings were taken using a digital thermometer, and were monitored during the first month of the study.

Blood samples for PCR genotypic analysis were collected on Isocode® kits (Schleicher & Schuell, Ecquevilly, France). Only failures after day 9 were analysed by PCR. The analysis was performed by the Shoklo Malaria Research Unit, Mae Sot, Thailand, according to a published method considering the three *P. falciparum* gene loci merozoite surface protein-1 (msp-1), merozoite surface protein-2 (msp-2) and glutamate rich protein (GLURP) (Brockman *et al.* 1999). Briefly, this method allows for comparison of the genotype of pre- (inclusion) and post-treatment (failure) infection pairs; pairs with identical genotypes are classified as recrudescences, and those with different genotypes as re-infections. Misclassification may, however, occur when, by chance, patients are re-infected with a strain having a genotype identical to that of the pre-treatment infection. The chance of this occurring in Bundi Bugyo was evaluated as follows. First, the three-locus genotypes of 106 randomly chosen samples were determined; the population frequency of each three-locus genotype was then calculated [= (number of samples with genotype X)/106]. This frequency also represented the probability [known as *P*(match)] that a patient could have been re-infected with a strain of identical genotype. For any of the three-locus genotypes found in the study population, *P*(match) was <0.03, namely within the accepted limit (0.05) stipulated by this method: identical-genotype pairs could thus reliably be classified as recrudescences, and vice versa.

Sample size

Sample sizes for the SP and AQ groups were determined applying a Type I error risk of 0.05, a power of 0.80 and a projected 10% loss to follow-up. Assuming 40% failure, and with a desired precision of 10%, 100 children were to be included in the SP group, while in the AQ group, assuming 10% failure, and with 7.5% precision, an intended sample size of 70 was set. Sample size in the CQ + SP group was arbitrarily set at 90 based on EANMAT standards (minimum, $n = 60$, based on lot quality assurance sampling; ideal, $n = 120$, based on classical sample size calculation) and the projected speed of enrolment.

Data entry and analysis

Data were double-entered and analysed per-protocol on EPIINFO 6.04b (CDC, Atlanta, 1996). The proportion of recrudescences found among PCR-analysed failures was applied to failures after day 9 for which no PCR result was available, and an extrapolated number of failures was obtained. The overall number of failures in each treatment group was thus calculated as ETF + LCF before or on day 9 + all other LCF or LPF confirmed or extrapolated as true recrudescences from the PCR analysis. Anaemia was defined as haemoglobin <11 g/dl. Percent gametocyte carriage was compared among study groups by a chi-square test.

Results

Between January and November 2002, 5901 children were screened, of whom 685 (11.6%) were positive for *P. falciparum*. Of these, 268 met inclusion criteria: 100 received SP, 78 AQ and 90 CQ + SP. Baseline characteristics (Table 1) were similar across treatment groups, with the exception of gametocyte carriage ($P < 0.01$). After inclusion, 3.7% (10/268) of children were lost to follow-up, and 7.1% (19/268) were withdrawn due to incorrect inclusion (5), intake of non-study antimalarials (4), vomiting dose twice (3), incorrect dosage (3), acute respiratory infection (2) and re-infection with a non-falciparum species (2).

Clinical failure proportions at day 14 (ETF + LCF days 4–14), without PCR adjustment, were highest in SP group (23.4%), compared to 8.8% and 6.0% in the AQ and CQ + SP groups, respectively (Table 2). After PCR adjustment, overall (ETF + LCF + LPF) failure proportions at day 28 increased considerably in all groups, but remained highest among SP patients (37.0%), whereas failure in the AQ and CQ + SP groups was similar (20.6% and 22.8%, respectively), and significantly lower than for SP ($P < 0.05$ for both SP *vs.* AQ and SP *vs.* CQ + SP comparisons). Early failures were also more frequent in the SP group ($P < 0.05$ for both comparisons). Parasite clearance on day 3 was only 56.3%

Table 1 Baseline (day 0) characteristics of included patients (*in vivo* antimalarial efficacy study, Bundi Bugyo, Uganda, 2002)

Characteristic	SP ($n = 100$)	AQ ($n = 78$)	CQ + SP ($n = 90$)
Age (months), mean (standard deviation)	25.3 (12.5)	19.7 (10.3)	24.2 (13.8)
Gender ratio (male/female)	0.87 (47/53)	1.36 (45/33)	1.00 (45/45)
Middle-upper arm circumference (mm), median (standard deviation)	142.1 (11.5)	141.9 (11.9)	145.1 (13.3)
Axillary temperature (°C), median (range)	37.4 (36.0–40.5)	37.7 (36.0–40.6)	37.4 (36.1–39.5)
Asexual parasitaemia (μl^{-1}), geometric mean (inter-quartile range)	10 057 (3932–28 256)	11 803 (3973–38 583)	11 317 (4101–29 385)
Gametocyte carriage, % (95% CI)	26.0 (17.7–35.7)	6.4 (2.1–14.3)	6.7 (2.5–13.9)
Haemoglobin (g/dl), median (range)	11.7 (5.3–15.3)	11.7 (6.0–15.3)	10.7 (6.7–14.0)

Table 2 Clinical failure proportions at day 14 (ETF + LCF, no PCR adjustment) and overall failure proportions at day 28 (ETF + LCF + LPF, after PCR adjustment) (*in vivo* antimalarial efficacy study, Bundi Bugyo, Uganda, 2002)

	Outcome				Clinical failure, day 14			Overall failure, day 28		
Day	ETF 1–3	LCF 4–14	LCF 15–28	LPF 28	*n*	%	95% CI	*n*	%	95% CI
SP	14	8	18	22	22/94	23.4	15.3–33.3	34/92*	37.0	27.1–47.7
AQ	0	6	18	5	6/68	8.8	3.3–18.2	14/68†	20.6	11.7–32.7
CQ + SP	3	2	18	12	5/84	6.0	2.0–13.3	18/79‡	22.8	14.1–33.6

* SP: 17 failures before or on day 9 + 15 PCR-confirmed (8 LCF, 7 LPF) + 5 extrapolated (LCF or LPF).
† AQ: 0 failures before or on day 9 + 11 PCR-confirmed (10 LCF, 1 LPF) + 3 extrapolated (LCF or LPF).
‡ CQ + SP: 3 failures before or on day 9 + 10 PCR-confirmed (9 LCF, 1 LPF) + 5 extrapolated (LCF or LPF).

(53/94) in the SP group, compared to 77.1% (54/70) in the AQ group and 74.7% (62/83) in the CQ + SP group ($P < 0.02$ for the same comparisons).

Baseline gametocyte carriage was 26.0% (26/100) in the SP group, reached 44.0% (43/80) on day 14 and remained at 25.4% (15/59) on day 28 among patients who were still being followed-up. Baseline carriage was lower and comparable in the AQ (5/78 or 6.4%) and CQ + SP (6/90 or 6.7%) groups. By day 14, however, it was only 1.5% (1/68) in the AQ group, compared to 25.9% (21/81) in the CQ + SP group ($P < 0.05$). Gametocytaemia in both groups was low on day 28 [0.0% (0/44) and 5.2% (3/58)].

Of 62 children anaemic on day 0 and re-assessed on day 28, 52 (83.9%) presented a haemoglobin level higher than baseline. Between day 4 and day 28, 70 children with reappearing parasites but no fever were followed-up without rescue treatment; of these, 46 (65.7%) developed fever (LCF), 20 (28.6%) remained afebrile but parasitaemic up to day 28 (LPF) and only four (5.7%) cleared parasites (ACPR). This pattern was similar in all treatment groups.

Discussion

This *in vivo* study provides fresh data on antimalarial efficacy in a border area of Uganda where information on resistance was scarce. Specifically, it offers new information on the performance of CQ + SP (Uganda's first-line antimalarial regimen) based on a PCR-adjusted 28-day follow-up, rather than the less ideal 14-day method.

The main limitation of this study was its non-randomized design, making a comparison of study regimens difficult: enrolment in the treatment groups occurred in different transmission seasons, with potentially different ecological balances of resistant strains. Different transmission intensity during enrolment of each study group should not have created a large bias in outcome, as PCR would have detected re-infections. Failure to randomize, nevertheless, means that inter-group comparisons should be interpreted with caution (this is particularly the case for gametocyte carriage, which was clearly higher at baseline in the SP group). Also, exclusion of children with a

previous intake of a full antimalarial course may have resulted in an underestimation of treatment failure, as patients highly suspect of harbouring a resistant strain were systematically left out. Malabsorption could have played a role in some failures: we did not attempt to measure drug concentrations in plasma, as this entails a lengthy procedure rarely performed in antimalarial efficacy monitoring. Enrolment in the study lasted 9 months: the proportion of children with confirmed malaria was lower than expected, suggesting that the disease's burden may have been overestimated in Bundi Bugyo.

Our results show that SP resistance has reached alarming levels in Bundi Bugyo, up nearly fivefold from 1997 considering day 14 clinical failure only. The proportion of early failures is particularly concerning. Addition of CQ to SP did seem to improve therapeutic response (notably in the early stage), mirroring findings from Kampala (Gasasira *et al.* 2003). However, the combination's overall efficacy was disappointing (23% failure), and already in the 'action' period requiring activities to identify a replacement therapy (WHO 2000b). Gametocyte carriage was also high after both SP and CQ + SP treatment, with important implications for community transmission of *P. falciparum,* and especially resistant strains (Sokhna *et al.* 2001; Bousema *et al.* 2003). In Bundi Bugyo, SP does not seem like a viable partner drug for a combination regimen. Elsewhere in Uganda, as SP resistance builds, similar trends may be expected (Talisuna *et al.* 2002).

In this study, moderate resistance to AQ (21%) was also noted, though no ETF and rapid gametocyte clearance were observed. In Bundi Bugyo, a combination of artesunate (AS) and AQ could prove efficacious (Adjuik *et al.* 2002), and, if used correctly, slow or reverse the development of resistance, as shown elsewhere (Nosten & Brasseur 2002). Artemether plus lumefantrine (Coartem®) could be a second option. Both of these artemisinin-based combinations, however, remain considerably more expensive than currently used regimens.

Methodologically, our *in vivo* study confirms the importance of extending follow-up to at least 28 days, as evidenced elsewhere in Uganda (Dorsey *et al.* 2002). In this setting, a follow-up of only 14 days would have seriously underestimated failure proportions. Our findings also demonstrate the relevance of parasitological (LPF) criteria of failure, recently added to the WHO classification: of children with asymptomatic parasitaemia during follow-up, two-thirds became symptomatic, and only 6% spontaneously eliminated their parasites, confirming results from Tanzania (Mutabingwa *et al.* 2001). Early and late recrudescences may be intrinsically different because of strain virulence or host immunity (White 2002). Nevertheless, our study suggests that, among children in a setting such as Bundi Bugyo, LPF represents merely the pre-clinical stage of the recrudescence process, and is thus a valid criterion of therapeutic failure.

In summary, this study from western Uganda highlights the need to prepare a change in the current first-line antimalarial protocol. We believe our findings discourage the future long-term deployment of SP containing combinations in this region, as their useful therapeutic lifespan is expected to be short. In Bundi Bugyo, an AS + AQ combination should be prioritized. Policy decisions should be based on *in vivo* studies with a follow-up of at least 28 days, PCR genotyping to detect reinfections, and both clinical and parasitological criteria of failure.

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