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Efficacy of sulphadoxine–pyrimethamine alone or combined with amodiaquine or chloroquine for the treatment of uncomplicated falciparum malaria in Ugandan children

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Summary The rapid development of falciparum resistance to sulphadoxine–pyrimethamine (SP) in East and Central Africa has raised concerns as to the efficacy of combining it with another drug. In 2002, we assessed the efficacy of SP alone and combined with amodiaquine (AQ/SP) or chloroquine (CQ/SP) in Ugandan children with uncomplicated falciparum malaria. At day 14, adequate clinical response was 100% (84/84) for AQ/SP, 93% (92/101) for CQ/SP and 91% (73/80) for SP. At day 28, parasitological failure (RI–RIII) occurred in 16% (13/80) of children treated with AQ/SP, in 48% (48/100) of those treated with CQ/SP and in 61% (48/79) of those treated with SP alone. Compared with the AQ/SP arm, the odds for parasitological failure at day 28 were five times higher (95% CI, 2–10) in the CQ/SP group and sevenfold higher (95% CI, 3–17) in that of SP alone. CQ/SP does not offer any significant added benefit over SP alone while AQ/SP is an efficacious low-cost combination. These findings have important policy implications for Uganda and other resource-constrained African countries faced with the problematic choice of a new first-line antimalarial treatment in a context of high CQ resistance.

keywords *in vivo* efficacy, sulphadoxine–pyrimethamine, chloroquine, amodiaquine, combination therapy, falciparum malaria, Uganda

Introduction

Malaria, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis and measles are among the leading infectious diseases in the world and mostly afflict the poorest populations. Although the mortality attributable to malaria worldwide is not exactly known, the greatest burden of the disease occurs in sub-Saharan Africa, mainly in children under 5 years of age and in pregnant women, particularly in primigravidae. In the past, severe ill health and death caused by malaria has been held in check by safe and affordable antimalarial drugs such as chloroquine (CQ) and sulphadoxine–pyrimethamine (SP). However, the rapid spread of drug resistance has worsened the burden of malaria. Resistance to SP, the next affordable drug after CQ, is rapidly developing in the eastern/Great Lakes, central and southern African regions and a public health disaster because of multi-drug resistant malaria has been predicted (Mutabingwa *et al.* 2001; Staedke *et al.* 2001; Dorsey *et al.* 2002; Kamya *et al.* 2002; Legros *et al.* 2002; Talisuna *et al.* 2002a). It is assumed that SP resistance appears more rapidly than CQ resistance because its half-life is long and persisting subtherapeutic concentrations would increase the chances of selecting resistant parasites (Watkins *et al.* 1997). The useful therapeutic life (UTL) for SP is likely to be short and this is already supported by field evidence from Uganda, a country where in 2001 CQ was still the first-line drug for the treatment of uncomplicated malaria. *In vivo* tests carried out in 1998 and 1999 in seven sites showed that the median SP parasitological failure (RI–RIII) after 14 days of follow-up was 20% (range: 0–73%) (Talisuna *et al.* 2002a). Similarly, in Kilifi, Kenya, a steady increase of SP parasitological failure has been observed in the last few years (Nzila *et al.* 2000), despite the low SP use.

On the basis of field evidence from South-east Asia, where combination therapy with artemisinin derivatives has maintained its efficacy and possibly delayed the spread of resistance (White 1999a; Nosten *et al.* 2000), the use of artemisinin derivatives has been proposed as a strategy to tackle the problem of drug-resistant falciparum malaria. Nevertheless, cost implications have precluded the quick adoption of artemisinin-based combinations in most African countries. Some of these, such as Uganda and Rwanda, have recently changed the first-line treatment from CQ monotherapy to CQ/SP and AQ/SP, respectively (Uganda Malaria Control Policy, 2001 unpublished report; Report of the Rwanda Consensus Meeting on the Antimalarial Drug Policy, 2001, unpublished report), a decision based mainly on economic rather than scientific considerations.

Several studies have reported the efficacy of combination therapies, but few have assessed the efficacy of low-cost combinations. Either, administration of cheap combinations were compared with monotherapy (Staedke *et al.* 2001; reviewed in McIntosh & Greenwood 1998) or combinations containing an artemisinin derivative were compared with monotherapy (Adjuik *et al.* 2002; Dorsey *et al.* 2002). Very few studies have compared the efficacy of multiple combinations without artemisinin derivatives in places with high CQ resistance and rapidly developing SP resistance. A study in Kampala compared the efficacy of AQ/SP with CQ/SP and SP alone in all age groups (Gasasira *et al.* 2003), but used a 14-day follow-up to determine treatment efficacy, and the limitations of this shorter period of follow-up justify our study. We investigated, using a 28-day follow-up, the efficacy of the same treatments in children aged 6–59 months in a rural area with a different pattern of transmission (hyper- to holoendemic) and drug use prevalence than Kampala.

Materials and methods

Study area

The study was conducted between December 2001 and March 2002 at the Nagongera Health Centre, in Tororo District in eastern Uganda, along the Kenyan border. Rainfall peaks from March to May and from August to September; the average temperature varies from 18 to 32 °C and the average humidity is 40–50%. Malaria is hyper- to holoendemic and the parasite prevalence in all ages and in children aged 2–9 years is 71% and 91%, respectively (Talisuna *et al.* 2002a). Chloroquine, SP, quinine and AQ are the most commonly used antimalarial drugs and can be found in government health units, drug shops, retail shops, kiosks and private clinics.

Treatment allocation and drug administration

This was an open label, alternate drug allocation study conducted according to strict guidelines from the East Africa Network for Monitoring Antimarial Treatment (EANMAT) and the World Health Organization protocol (WHO/CTD 1996) for areas of intense transmission. The first 150 consecutive patients who satisfied the inclusion criteria were alternately allocated according to the following predetermined order: CQ/SP, AQ/SP and SP monotherapy. Subsequently, patients were alternately allocated as follows: two to the CQ/SP arm and one to AQ/SP or SP monotherapy. This allocation scheme was decided before the study started, in order to achieve the required number of study participants in the CQ/SP arm for a parallel molecular study. Children aged 6–59 months with fever (axillary temperature ≥37.5 °C) or history of fever in the past 24 h, *Plasmodium falciparum* monoinfection and a parasite density between 2000 and 100 000/μl were recruited, treated and followed up for 28 days after treatment. Children with general danger signs or severe malaria, other causes of fever, severe malnutrition and a history of allergic reactions to sulpha drugs were excluded. A regional quality control laboratory in Kenya verified the quality of all test drugs. Patients received either a single dose of SP monotherapy (25 mg/kg sulphadoxine and 1.25 mg/kg pyrimethamine) or a single dose of SP plus CQ (25 mg/kg, in three divided doses on days 0, 1 and 2) or a single dose of SP plus AQ (25 mg/kg, in three divided doses on days 0, 1 and 2). All drugs were administered orally under the direct supervision of a study nurse and according to the body weight. Children were observed for 30 min after treatment and a dose was re-administered if they vomited. All patients were given paracetamol for 2 days. Besides the day of recruitment, patients were seen again on days 1, 2, 3, 7, 14, 21 and 28 and on any other day if they were sick. Patients who did not turn up for a scheduled visit were actively followed up. Children lost during follow-up, those who took drugs from other sources or developed concomitant infections were censured in the intent-to-treat analysis and were not included in the analysis as per protocol.

Outcome measurements

The primary outcomes were clinical or parasitological responses. Clinical response was classified as follows: early treatment failure (ETF), late treatment failure (LTF) and adequate clinical response (ACR) as defined previously (WHO/CTD 1996). The ACR classification has been criticized and a recent WHO expert committee (WHO 2001) recommended that future studies should not use it

because it includes patients who have parasites at day 14 but have no fever. However, we have conducted the present study using the 1996 WHO protocol and used the ACR classification to enable comparison with previous studies. For the parasitological response the standard S–RI–RII–RIII classification system was used (WHO 1965). Furthermore, we used other secondary end points such as the acceptable parasite clearance rate for each treatment arm (i.e. proportion of patients who had acceptable parasite clearance and remained parasite-free during follow-up). On day 3, the acceptable parasite clearance was defined as a parasite density <25% of that at day 0, while on days 7, 14, 21 and 28, the acceptable parasite clearance was defined as absence of parasitaemia. Haemoglobin (Hb) was measured at day 0 and at days 14 and 28.

Laboratory investigations

Duplicate thick and thin blood films were collected on days 0, 3, 7, 14, 21 and 28 and on any unscheduled visit. The presence of *P. falciparum* in the peripheral blood was determined by microscopic examination of 100 high-power oil immersion fields. Asexual parasites were counted against 200 white blood cells (WBCs) and parasite density was computed assuming 8000 WBC/μl. All the slides from the study participants who had reached an outcome were blindly re-examined by an independent microscopist in Uganda and no discrepancies in the classification of outcomes were observed.

Distinguishing recrudescence from new *P. falciparum* infections

Blood samples for parasite genotyping were collected onto 3 MM Whatman filter paper from each study subject for all scheduled and unscheduled visits and stored in individual zip-lock polythene bags at room temperature. DNA was extracted by the Chelex-100 method (Plowe *et al.* 1997) and was used immediately or stored at −80 °C for later amplification. Genotyping was carried out at the merozoite surface protein-1 (*MSP1*) and merozoite surface protein-2 (*MSP2*) gene loci on DNA obtained from parasite isolates on day 0 (pre-treatment) and on the day of parasite reappearance (post-treatment) according to the techniques described previously (Ranford-Cartwright *et al.* 1997). Using digital imaging software, a new infection (strain) was recorded if the difference in fragment size in the post-treatment sample exceeded that of the pre-treatment sample by more than 20 base pairs (bp). Three categories of infections were identified using the above criterion: only recrudescent infections, both recrudescent and new infections and only new infections; the first two were considered to be recrudescence for the analysis.

Statistical analysis

The sample size was estimated assuming a prevalence of treatment failure for SP or CQ/SP of 15% and that for AQ/SP of approximately 1%. These estimates were based on previous *in vivo* tests for SP and AQ in Uganda (Staedke *et al.* 2001; Talisuna *et al.* 2002a). A minimum of 72 children per treatment group were needed to detect a significant difference between SP or CQ/SP and AQ/SP at the 95% confidence level and 80% power. Before analysis, data were checked for consistency using Stata 7.0 (Stata Corporation, College Station, TX). Analysis was performed both by intent-to-treat using a clinical life table with actuarial adjustment and as per protocol. Differences in the categorical outcomes of interest were tested using the chi-square test. Normally distributed continuous variables were compared using analysis of variance (ANOVA) and non-normally distributed continuous data were normalized by logarithmic transformation. In the analysis per protocol, we conducted multivariate analysis to assess the risk of failure for SP or CQ/SP when compared with AQ/SP. The odds ratios (OR) and the 95% confidence intervals were computed and a two-tailed *P*-value ≤0.05 was considered significant.

Results

Among the 740 screened patients, 460 (62%) were excluded for several reasons (danger signs, residence outside the designated area, parasite density outside the required range, or presence of concomitant infections) (Figure 1). The remaining 280 children were allocated either to AQ/SP (87, 31%), CQ/SP (105, 38%) or SP (88, 31%). Most were followed up to 14 (265, 95%) and 28 (259, 93%) days. The treatment groups were similar for key baseline demographic, anthropometric, haematological and parasitological characteristics (Table 1). However, more children in the AQ/SP arm reported that they had taken an antimalarial drug prior to recruitment, although the difference was not statistically significant.

At day 14, treatment failure (early or late) occurred in none of the children in the AQ/SP-treated group, in 8% (8/101) of those treated with CQ/SP and in 9% (7/80) of those treated with SP. Clinical response in the AQ/SP arm was significantly better than that for CQ/SP ($P = 0.01$) or SP alone ($P = 0.008$). Most (6/7) of the treatment failures in the SP monotherapy arm occurred in the first 3 days, while in the CQ/SP arm, only two of eight occurred in the first 3 days ($P = 0.2$) (Table 2). Parasitological failure

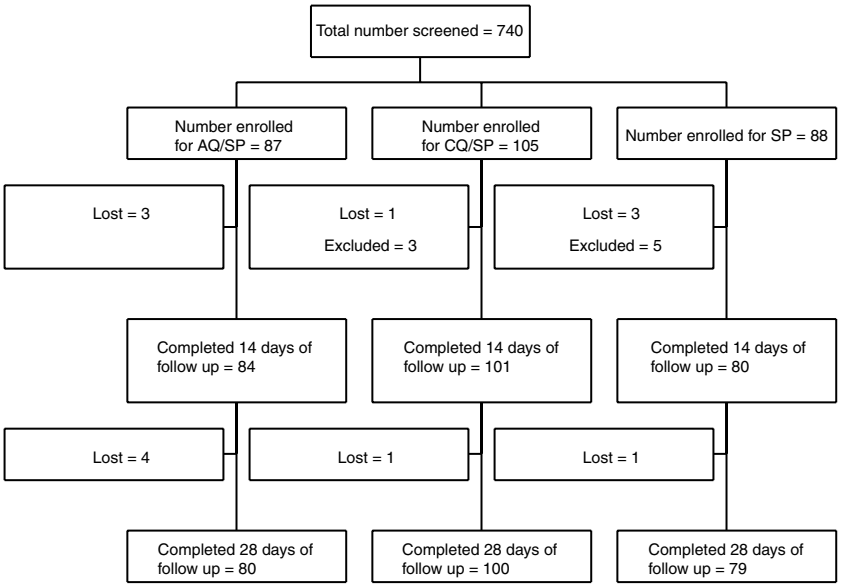

Figure 1 Trial profile. Shows the recruitment scheme and patients excluded or lost during 14 or 28 days of follow-up.

Table 1 Baseline characteristics by treatment group

Characteristic	AQ/SP ($n = 87$)	CQ/SP ($n = 105$)	SP ($n = 88$)
Mean age in months (SD)	18.5 (10.5)	19.9 (10.2)	18.4 (8.8)
Mean weight in kg (SD)	9.1 (2.0)	9.7 (2.3)	9.4 (2.2)
Mean haemoglobin in g/dl at day 0 (SD)	7.8 (1.3)	7.7 (1.2)	7.6 (1.0)
Mean temperature at day 0 in °C (SD)	38.2 (1.0)	37.8 (0.93)	37.5 (0.8)
Geometric mean parasite density assuming 8000 WBC/μl at day 0 (range)	14 346 (1440–99 120)	14 267 (1600–97 600)	10 725 (2240–97 279)
Proportion with history of treatment for malaria at enrollment [*n* (%)]	15 (17.2%)	9 (10.6%)	10 (11.3%)
Proportion that reported fever 48 h prior to enrollment [*n* (%)]	82 (94.3%)	96 (91.4%)	81 (92.1%)
Evaluable patients for day 14 assessment	84	101	80
Evaluable patients for day 28 assessment	80	100	79

during the 28 days of follow-up was significantly higher in the CQ/SP and the SP arms than in the AQ/SP arm ($P < 0.001$) (Table 2).

Of the 109 treatment failures, eight were ETFs. ETFs are caused by a failure of the drug to eliminate the initially detected (probably resistant) parasite strains and should ideally be excluded from genotyping. However, we included these eight paired samples as an internal control and were able to successfully genotype all 109 paired samples (pre- and post-treatment), 94 (86%) of which were classified as recrudescence while only 15 (14%) were new infections. The estimates of parasitological failure when excluding the new infections did not change the results significantly; AQ/SP performed significantly better than the other two treatments (Table 2). The odds for parasitological failure during 28 days of follow-up with the AQ/SP arm as reference were 4.6-fold higher (95% CI 2.1–10.1) in the CQ/SP group ($P < 0.001$) and 7.4-fold higher (95% CI 3.2–17.1) in the SP group ($P < 0.001$).

We used a clinical life table to determine the acceptable parasite clearance rate for each treatment group during 28 days of follow-up. The acceptable parasite clearance rate at days 3, 7, 14 was not significantly different amongst the three groups (Figure 2). However, with a longer period of follow-up (beyond 14 days), the acceptable parasite clearance rate declined significantly in the CQ/SP- ($P = 0.0004$) and SP- ($P = 0.0001$) treated groups, but not for the AQ/SP-treated group.

Table 2 Analysis per protocol using the standard WHO/CTD 1996 classifications

	AQ/SP ($n = 84$)		CQ/SP ($n = 101$)		SP ($n = 80$)	
Treatment outcomes at day 14	Number (%)	95% CI	Number (%)	95% CI	Number (%)	95% CI
Adequate clinical response (ACR)	84 (100)	96–100	93 (92)	85–97	73 (91)	83–96
Early treatment failure (ETF)	0 (0)	0–4	2 (2)	0.2–7	6 (8)	3–16
Late treatment failure (LTF)	0 (0)	0–4	6 (6)	2–13	1 (1)	0.03–7
Total treatment failure (TTF)	0 (0)	0–4	8 (8)	4–15	7 (9)	3–17
Parasitological outcomes at day 28*						
Sensitive (S)	67 (84)	74–91	52 (52)	42–62	31 (39)	28–51
RI	13 (16)	9–26	47 (47)	37–57	44 (56)	44–67
RII	0 (0)	0–5	0 (0)	0–4	2 (3)	0.3–9
RIII	0 (0)	0–5	1 (1)	0.02–5	2 (3)	0.3–9
Total parasitological failure (TPF)	13 (16)	9–26	48 (48)	38–58	48 (61)	49–72
Total parasitological failure (TPF)†	12 (15)	8–25	40 (40)	30–50	42 (58)	41–64

AQ/SP, amodiaquine/sulphadoxine–pyrimethamine; CQ/SP, chloroquine/sulphadoxine–pyrimethamine; SP, sulphadoxine–pyrimethamine.
* Patients lost during follow-up between day 14 and 28: AQ/SP = 4, CQ/SP = 1, SP = 1.
† Parasitological failure excluding the new infections (one, eight and six new infections occurred in the AQ/SP, CQ/SP arm and SP arm, respectively.

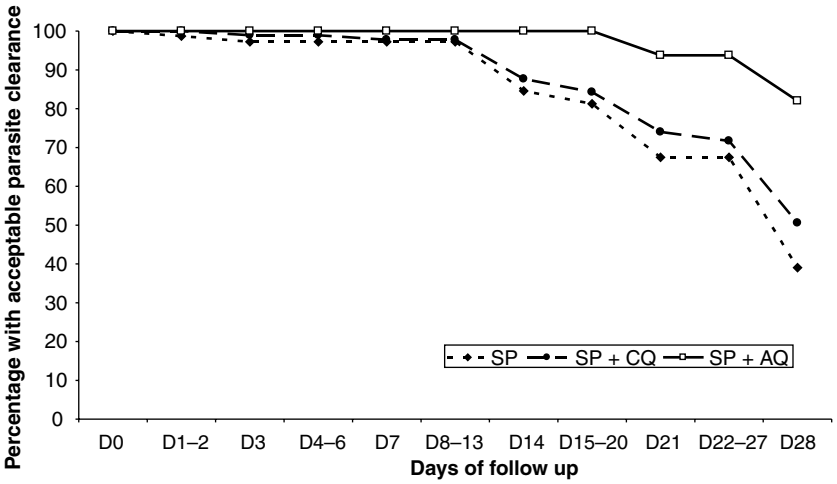

Figure 2 Acceptable parasite clearance rate by treatment group. A clinical life table with actuarial adjustment was used to determine the acceptable parasite clearance rate (i.e. proportion of patients with who had acceptable parasite clearance and maintained that status during 28 days of follow-up). Acceptable parasite clearance is defined in the 'Methods' section.

Haematological recovery was observed in all treatment arms and was most pronounced in the first 14 days of follow-up (Figure 3). There were no significant differences between the three groups ($P = 0.5$) (Figure 3).

Discussion

In this three-arm study, AQ/SP was significantly more efficacious than CQ/SP or SP monotherapy. Furthermore, CQ/SP did not offer any significant added benefit over SP monotherapy. These findings confirm that countries such as Uganda that adopted, albeit without evidence, the CQ/SP option as an interim first-line regimen to replace CQ monotherapy, may have to re-evaluate their decision very soon. Our data add weight to the early but growing evidence that an interim drug policy based on CQ/SP is probably a poor choice. Indeed this policy has stimulated considerable debate between those reluctant to change on the basis that the distribution of CQ resistance is heterogeneous and those in favour of a more radical change in drug policy despite the absence of adequate data on the efficacy of CQ/SP. Nevertheless, a recent trial in Uganda's capital town, Kampala, demonstrated that AQ/SP was more efficacious than CQ/SP and the latter was only marginally more efficacious than SP monotherapy (Gasasira *et al.* 2003). We have conducted our study in a rural setting with hyper- to holoendemic malaria and with a different pattern

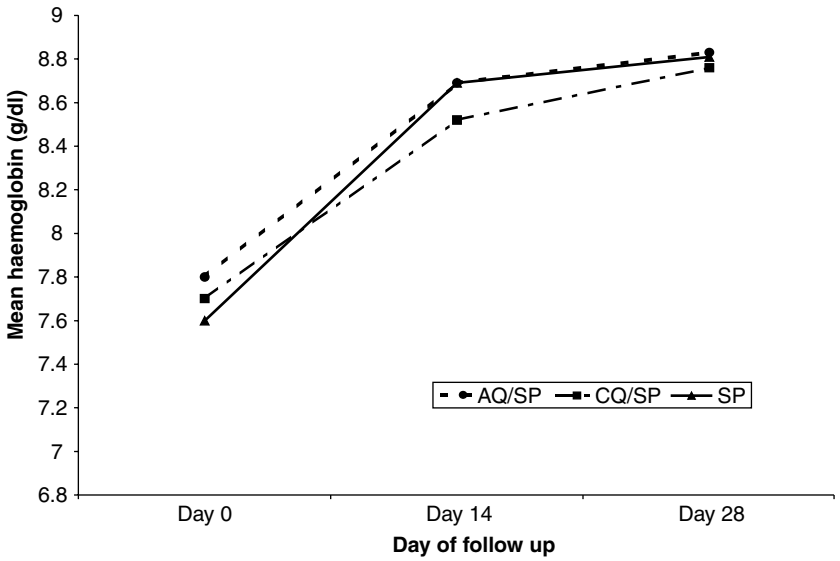

Figure 3 Haematological recovery by test drug (haemoglobin estimate in g/dl).

of drug use and have confirmed that CQ/SP is not appropriate for areas with high CQ resistance and increasing SP resistance.

Combination therapy, well established as a strategy in the management of cancer, tuberculosis, leprosy and HIV/AIDS, has been proposed as a possible solution to contain the problem of antimalarial drug resistance. This is based on the notion that it is less likely to have resistance to both drugs than to individual drugs because the former is the product of the resistance mutation frequency for the individual drugs (White 1999b; Hastings & D'Alessandro 2000). However, a key issue in combination therapy is the drug elimination half-life of the partner drugs. Sulphadoxine–pyrimethamine and AQ have a long and matched drug elimination half-life of about 4–6 days. Combinations involving drugs with a matched half-life probably reduce the chances that parasites will be exposed to suboptimal (selective) levels of one drug once the partner drug with a short half-life has been eliminated. The matched elimination half-life for AQ and SP would probably result in the protection of the partner drugs in the combination.

We have demonstrated that the acceptable parasite clearance rate is not significantly different in the first 2 weeks. However, there is a remarkable decline in the efficacy of CQ/SP and SP monotherapy between days 14 and 28. Moreover, molecular data suggest that most infections identified during this period were incidents of recrudescence and not new infections. These findings confirm that a period of follow-up of only 14 days underestimates the true prevalence of treatment failure. Such underestimation is likely to be more pronounced in combination therapy that involves drugs with a long half-life such as CQ/SP and AQ/SP, but could also occur for short half-life antimalarials. Similar observations have already been reported by other studies such as the recent one in Uganda that followed up patients for more than 42 days (Dorsey *et al.* 2002). These observations taken together imply that the follow-up period for *in vivo* tests, especially those involving combinations of drugs with a long elimination half-life should be at least 28 days.

Although our data suggest that the short-term efficacy of AQ/SP is good, its long-term efficacy remains unknown. This brings into sharp focus its deployment as an interim policy pending a better long-term option. In view of the high cost of the artemisinin derivatives, a combination involving SP and AQ, which are affordable drugs and have a matched half-life, is probably a better interim policy option than CQ/SP for African countries with intense malaria transmission. Furthermore, in the absence of long-term efficacy data, mathematical models remain the only option for offering insights on the evolution of resistance and such models suggest that the long-term efficacy of AQ/SP might be short (I.M. Hastings and W.M. Watkins, personal communication). Whether such predictions are correct can only be established after AQ/SP has been used for some time. Recent studies in Uganda and Rwanda (Dorsey *et al.* 2002; Rwagacondo *et al.* 2003) have demonstrated that SP plus artesunate (SP/ART) is less efficacious than AQ/SP, particularly when the period of follow-up is extended beyond 14 days. This observation is probably related to the lower efficacy of SP in these settings, lack of efficacy of a 3-day course of artesunate and the absence of synergy of both drugs *in vitro*. In areas of intense transmission, the long elimination half-life of SP

and AQ could increase the period over which new infecting parasites (sensitive, partially and fully resistant) are exposed to residual drug concentrations. In our study setting, the ideal combinations are likely to be those involving drugs with a matched and short elimination half-life such as artesunate plus chlorproguanil-dapsone (LapDap). However, LapDap has been registered only recently and its combination with artesunate will require toxicity studies that are not likely to be completed within the next 2–3 years. Therefore, efficacious interim policies such as AQ/SP may be warranted.

The magnitude of therapeutic failure at which a partner drug ceases to improve the efficacy of the combination is not known. However, our data demonstrate that a drug with a low therapeutic efficacy such as CQ in this study setting, 44% treatment failure at day 14 (Talisuna *et al.* 2002a), does not improve the efficacy of the combination. Moreover, the prevalence of infections with the *P. falciparum* chloroquine-related transporter (*pfcrt)* gene mutations linked to CQ resistance is approximately 100% among children visiting the local health facility (Kyosiimire-Lugemwa *et al.* 2002) and about 90% in the general population (Talisuna *et al.* 2002b). These observations suggest that mutations linked to CQ resistance are saturated in this area and any combination involving CQ is likely to fail very soon. However, our data also suggest that SP treatment efficacy is low (day 28 polymerase chain reaction (PCR)-adjusted efficacy of only 42%), while the day 28 PCR-adjusted efficacy of AQ/SP is 85%. The higher efficacy of AQ/SP is probably because of the good performance of AQ alone. In the absence of an AQ-alone study arm, it is not clear from the present study how much of the superior efficacy of AQ/SP is due to the combination. Moreover, the prevalence of infections with three dihydrofolate reductase (*DHFR)* mutations at codons 108, 51 and 59 is over 80% in this study setting (Talisuna *et al.* 2003). Furthermore, the ready availability of both SP and AQ as monotherapy is a major factor that could limit the therapeutic life of the AQ/SP combination. This raises concerns about the long-term efficacy of any combination involving SP in this setting.

A limitation of our study is that we used alternate allocation of patients to the different treatment arms instead of using the generation of a pre-study random list. Randomization is the ideal method for concealment of treatment allocation. Alternate allocation schemes are simple but they are prone to bias. We have attempted to limit such bias by determining the treatment allocation scheme before the study commenced and designating only one study nurse to strictly adhere to the predetermined alternate allocation of the treatment.

Our study confirms that AQ/SP is an efficacious treatment option for non-complicated malaria and could be used as an interim policy in resource-constrained countries that already have high CQ resistance prevalence. However, the idea of interim policies although attractive, may result in the deployment of a chain of suboptimal options. Consequently our data could be used in resource-constrained countries that have high CQ and SP resistance to adopt an efficacious artemisinin combination therapy without a holding measure of an interim policy. Finally, our study confirms that areas with a high prevalence of CQ resistance like that found in our setting should avoid using CQ as a partner drug in any combination therapy.

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