COMPARISON OF THE PARASITOLOGIC EFFICACY OF AMODIAQUINE AND SULFADOXINE-PYRIMETHAMINE IN THE TREATMENT OF Plasmodium Falciparum MALARIA IN THE BUNGOMA DISTRICT OF WESTERN KENYA


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Abstract. The efficacy of amodiaquine (AQ) and sulfadoxine-pyrimethamine (SP) was assessed in 310 symptomatic children from western Kenya with uncomplicated Plasmodium falciparum malaria. A non-blinded, randomized, 14-day study was performed and parasitologic criteria were used. Of 310 patients included, 238 (77%) completed the study: 120 received AQ and 118 received SP. In those treated with AQ, there were sensitive (S) infections in 107 patients (89.2%, 95% confidence interval [CI] = 82.2, 94.1%), RI resistance in 10 (8.3%, 95% CI = 4.1, 14.8%), RII resistance in 1 (0.8%, 95% CI = 0, 4.6%), and RIII resistance in 2 (1.7%, 95% CI = 0.2, 5.9%). In those treated with SP, there were S infections in 74 patients (62.7%, 95% CI = 53.3, 71.4%), RI resistance in 21 (17.8%, 95% CI = 11.4, 25.9%), RII resistance in 11 (9.3%, 95% CI = 4.7, 16.1%), and RIII resistance in 12 (10.2%, 95% CI = 5.4, 17.1%). Resistance rates were consistently higher in the SP-treated patients (P < 0.001). Resistance to SP in this area has reached such levels that it should no longer be the first-line treatment. Alternative treatment, such as SP plus AQ combination treatment or artemisinin combination treatment, is urgently needed.

INTRODUCTION

Malaria is one of the most important causes of morbidity and mortality in Africa. It is estimated that 0.5–2.5 million persons die due to malaria and that 300–500 million clinical cases occur yearly.1 Epidemiologic data suggest that malaria mortality has increased since 1984 because of increasing chloroquine (CQ) resistance of Plasmodium falciparum.7 Several east African countries have adopted sulfadoxine-pyrimethamine (SP) as first-line treatment for uncomplicated P. falciparum malaria instead of CQ.1 It was feared that resistance to SP would develop relatively quickly because of its long elimination half-life,3 and this did occur in Kenya 4,5 and Tanzania,5–7 as it had previously occurred in Thailand.8

In Kenya, resistance to CQ was first detected in non-immune tourists in 1978 and in semi-immune Kenyans in 1982 (for references see Shretta and others9). In the 1990s, the percentage of resistance increased to 85% in western Kenya.9 In 1998, SP replaced CQ for first-line treatment and amodiaquine (AQ) became the drug for second-line treatment.9 Early studies showed good results for SP treatment, but resistance developed rather quickly, and in recent years cure rates varying from 50% to 90% have been reported from different endemic areas in Kenya.4,10–18 A study of SP efficacy conducted in 1999–2000 in Nairobi, a non-endemic area where reinfections hardly ever occur, showed 65% sensitive (S) infections, 27% with an RI response, and 8% with RII-III responses.19 In vitro results are consistent with these in vivo data; increased resistance of parasites to SP or one of its components was demonstrated in studies on the Kenyan coast.20 For AQ, variable results have been reported from various countries.21 In Kenya, where AQ was only introduced as a second-line drug, efficacy varied from 65% to 88%,17,19 even up to 97% (Cobelens FG, unpublished data),22 but Keuter and others, who used a relatively low dose (25 mg/kg) of AQ, reported a high (67%) resistance rate.23 Recent studies with a dose of 30 mg/kg showed cure rates of 74% at 14 days and only 54% at 28 days after correction by a polymerase chain reaction (PCR) for reinfections in Kenya, but higher cure rates in Senegal and Gabon at 28 days of 81% (not corrected) and 85% (PCR corrected), respectively.23 In Uganda, parasitologic cure rates after the use of SP, AQ, and the combination of SP plus AQ were 70–74%, 84%, and 90–99%, respectively.24,25 High levels of SP resistance have been reported in Tanzania.5–7,26 The East African Network for Monitoring Antimalarial Treatment, founded in 1998 and now including Uganda, Kenya, Tanzania, Zanzibar, Rwanda, and Burundi, recently published data on CQ, AQ, and SP from many sentinel sites, which confirmed the high failure rates of CQ, the emergence of SP resistance, and the still good performance of AQ.5

As part of the continuous monitoring of drug resistance in Kenya by the African Medical and Research Foundation (AMREF) in Nairobi4,12 we compared the efficacy of SP and AQ in the treatment of uncomplicated P. falciparum malaria in symptomatic children in the Bungoma District of western Kenya, an area with intense malaria transmission. Previous data on drug resistance/sensitivity from this area were not available.

MATERIALS AND METHODS

Study area. The study was conducted between April and June (rainy season) 2000 in the Bungoma District of western Kenya, an area of intense malaria transmission near Lake Victoria. The study sites were the Bungoma District Hospital, the Webuye Panpaper Clinic, and the Sirisia Health Center. The study was reviewed and approved by the Institutional Review Board of the Academic Medical Center (Amsterdam) and the Review Board of the African Medical and Research Foundation (Nairobi).

Patients and recruitment. Symptomatic outpatients 6–59 months of age with a positive blood smear were enrolled after informed consent of their parents or guardians was obtained, provided that they had an uncomplicated monoinfection with P. falciparum. Inclusion criteria were a parasitemia between 2,000/mm³ and 250,000/mm³, a body weight ≥ 5 kg, a history of fever in the preceding 24 hours or an axillary temperature ≥ 37.5°C at presentation, an ability to drink, and residence within a pre-determined perimeter from health center/
hospital. Exclusion criteria were vomiting two or more times in the preceding 24 hours, one or more convulsions in the preceding 24 hours, an inability to sit up or stand, a hemoglobin level < 5 g/dL, and signs of severe malaria, other febrile diseases, underlying disease (cardiac, renal, or hepatic), or a history of allergic reactions to AQ, SP, or other sulfa compounds.

Randomization and treatment. Treatment efficacy of AQ and SP was assessed in a 14-day, randomized, non-blinded, clinical trial. Treatment allocation was done in an unannounced fashion by the researchers by taking the next number from a randomization list (one for each center) that was prepared by one of us (FGC) before the start of the study from a random number table. Block randomization was applied to safeguard balance in numbers (blocks of 10). The AQ group received amodiaquine (Camoquin®, 200-mg tablets: Parke-Davis, Detroit, MI), 10 mg/kg of body weight, given orally on days 0, 1, and 2, and SP group received sulfadoxine/pyrimethamine (Fansidar®, 500 mg of sulfadoxine and 25 mg of pyrimethamine per tablet; Roche, Basel, Switzerland), 25 mg of sulfadoxine/kg of body weight, given orally once on day 0. Treatment was given under supervision and any dose vomited within an hour was repeated. Patients who vomited more than once were excluded from follow-up. Patients with a body temperature (axillary) > 38°C were given paracetamol syrup in accordance with local policy.

Patient follow-up. After recruitment (day 0), patients were seen again on days 1, 2, 3, 7, and 14 and on any other day in between if fever and/or other symptoms developed. On each occasion the clinical status was assessed and a thick blood smear was prepared to determine the asexual parasite density. The temperature was measured on days 0, 1, 2, and 3 and whenever clinically indicated. Patients who did not return for follow-up were visited at home.

Study endpoints were based on parasitologic response, which was up to then the procedure used in the monitoring by the AMREF. Grade III resistance (RIII) was a parasitemia on day 2 ≥ 25% of the initial (day 0) value. If no data for day 2 were available, data for day 3 were used instead. Grade II resistance (RII) was a parasitemia on day 7 in patients without grade III resistance. Grade I resistance (RI) was a parasitemia on day 14 in patients without grade II or III resistance. Sensitive (S) was no grade I, II, or III resistance.

Patients were withdrawn from the study if an endpoint was reached and were then given rescue treatment. Rescue treatment was parenteral quinine for all those with parasitologic failure in the presence of fever and oral SP both after AQ and after SP treatment for those without fever. This was according to local practice where AQ and other antimalarial drugs were not normally available.

Lost to evaluation includes those patients who were withdrawn at a parent’s or guardian’s request, those who were given antimalarial or antibiotic therapy outside the protocol, or those who did not return for evaluation for unknown cause and who could not be found at home. In addition, due to misunderstandings regarding the study protocol, some patients were given rescue treatment without reaching a study endpoint and they were not followed-up further. They are categorized as protocol violations.

Laboratory methods. Blood was obtained by finger prick for thin and thick blood smears. The slide was fixed and stained using Field’s stain. Parasite density was calculated by counting the number of asexual parasites per 200 leukocytes. As many fields as necessary were examined to find either 200 leukocytes or 500 parasites. The parasitemia was calculated on the assumption of a leukocyte count of 8,000/μL. Parasite density was assessed from the thick smear. Thin blood smears were reviewed for species identification. In Sirisia, the hemoglobin concentration was measured with a photometer (Combur 1000®, Bayer Diagnostics and Electronic Gmbh, Munich, Germany) after mixing 20 μL of finger prick blood with 5 mL of Drabkin solution. In Webye and Bungoma, hemoglobin was assessed by the HemoCue® procedure (HemoCue Ltd., Derbyshire, United Kingdom).

Statistical methods and analysis. Data were recorded on case record forms and entered twice in an automated database (Epi-Info version 6.0; World Health Organization, Geneva, Switzerland and Centers for Disease Control and Prevention, Atlanta, GA). Analyses were performed using STATA version 6.0 (StatCorp College Station, TX). The analysis was restricted to patients for whom parasitologic data were available on days 2, 7, and 14. If data were missing for day 2 but available for day 3, the latter were used instead. To assess potential bias by differential loss to follow-up between the treatment groups, a worst-case analysis was done in which all patients for whom parasitologic data were missing were assumed to have been resistant. Resistance grades were allocated such that patients who were lost to follow-up before grade III resistance could be ascertained were classified as RII, the remaining patients who were lost to follow-up before grade II resistance could be ascertained were classified as RI, and the remaining patients who were lost to follow-up were classified as RI.

Exact binomial confidence intervals (CIs) were calculated. For comparison of categorical variables, the chi-square test with Yates’ continuity correction for 2 × 2 tables or the two-sided Fisher’s exact test were used as appropriate. All tests were done at a significance level of $P = 0.05$.

RESULTS

After screening, 310 patients were included in the study and randomized to one of the treatment groups. The groups were comparable with respect to their baseline characteristics (Table 1). One hundred fifty-six were randomized to receive AQ and 154 to receive SP. The numbers of patients enrolled who did not complete the study were similar in the two treatment groups both as a whole and when stratified for the reasons they were excluded after enrollment (Figure 1). Of 310 patients, 238 (77%) completed the study. One patient with an RIII response to SP developed signs of severe malaria and died; none of the other patients with RI or RIII response developed signs of severe malaria.

Sensitive infections were present in 107 (89.2%) patients treated with AQ, and in only 74 (62.7%) patients treated with SP ($P < 0.001$). For all three grades, resistance rates were higher in the SP group than in the AQ group ($P < 0.001$) (Table 2). High-grade resistance (RII-III) was observed in 23 of 118 patients (19.5%) receiving SP versus 3 of 120 patients (2.5%) receiving AQ ($P < 0.001$).

In the worst-case analysis, the proportion of sensitive infections was again higher in the AQ group than in the SP group: 68.6% (107 of 156) versus 48.0% (54 of 154; $P < 0.001$),
and resistance rates were consistently lower across the three resistance grades (P = 0.003). In this analysis, high-grade resistance (RII-III) occurred in 50 of 154 patients (32.5%) receiving SP versus 33 of 156 patients (21.2%) receiving AQ (P = 0.025).

**DISCUSSION**

In Kenya, resistance to SP, the first-line treatment for malaria, has reached high levels in many, but not all, areas. This study showed a low sensitivity rate of 62.7% (95% CI = 53.3, 74.1%) for SP in the treatment of non-severe *P. falciparum* malaria in children in a high-transmission area in western Kenya. Sensitivity to AQ was good: 89.2% (95% CI = 82.2, 94.1%). The sensitivity to either drug may have been overestimated by this primary analysis since some of the patients who were lost to evaluation may in fact have been failures. We therefore included a worst-case analysis, in which it was assumed that all patients lost to evaluation had resistant parasites. This analysis thus provides the minimum sensitivity and maximum resistance that is compatible with our data. For sensitivity to AQ this was 68.6%, and for sensitivity to SP this was 48.0%, which is a statistically significant difference. Overestimation of drug sensitivity by our primary analysis may have occurred in patients who received parental treatment outside the study protocol or who were withdrawn for reasons not consistent with the study protocol (protocol violations). Since both were more frequent in the SP group than in the AQ group, this underestimation of sensitivity is likely to be more pronounced for SP than for AQ. It has to be mentioned that the 14-day follow-up period may also underestimate the true (28-day) risk of treatment failure.

Grade III resistance to SP was approximately as high as grade II resistance (10.2% and 9.3%, respectively; Table 2). However, this may be an overestimation since SP is a relatively slowly acting drug; patients may continue to clear parasitemia without further treatment, and may become cured, as has been observed after treatment with CQ. High-grade resistance (RII-III) to SP was significantly more frequent than that to AQ, both in the primary and in the worst-case analysis.

Several explanations can be made for the differences in resistance to SP and AQ. First, SP has a long elimination half life, while AQ is a pro-drug that produces metabolites with shorter half-lives. Resistance will develop more quickly to SP than for AQ. It has to be mentioned that the 14-day follow-up period may also underestimate the true (28-day) risk of treatment failure.

Finally, co-trimoxazole is commonly used in Kenya for the treatment of fever of unknown origin, for chest problems, and bacterial infections. One component of this drug, sulfamethoxazole, is a sulfa-drug similar to sulfadoxine and the other component, trimethoprim, is an analog of pyrimethamine. There is evidence of cross-resistance between pyrimethamine and trimethoprim, as well as between sulfadoxine and sulfamethoxazole. Terlouw and others demonstrated that treatment history (recent use of SP) and treatment dose (dosing based on age, not on actual body weight) were important determinants of SP efficacy, but after increasing the dose in their study area in western Kenya, the proportion of treatment failures continued to increase. They emphasize that adequate doses must be given from the start of deployment of an antimalarial drug in an area. In our study, since dosing was based on actual body weight, underdosing was not the cause of the high failure rate.
Combining drugs with different modes of action and mechanism of resistance is advocated to improve efficacy and delay development and spread of resistance. Combinations with an artemisinin drug are preferred and have also been studied in Africa. These artemisinin-based combinations are not yet available everywhere on a large scale and are more expensive than monotherapy and other combinations such as SP-CQ or SP-AQ. A Cochrane analysis of combination treatments of SP-CQ and SP-AQ showed superiority of the combination in comparison with monotherapy with regard to sustained parasite clearance and clinical improvement.

In Uganda, the current first-line treatment is SP-CQ based on studies comparing SP, AQ, and combinations of SP with CQ or AQ. Conversely, Rwanda opted for SP-AQ on studies comparing SP, AQ, and combinations of SP with sulfadoxine. In Kenya, the first-line treatment is still SP, but in some areas such as the Bungoma District, resistance to SP has reached levels that require reconsideration or even change of policy. A change of policy is a difficult and time-consuming process, and before decisions are made, let alone implemented, resistance may have increased to such a degree that a combination with another drug may no longer be useful. This may already be true in some areas in Kenya such as the Bungoma District where this study was performed. Continued use of SP monotherapy in an area of at least 40% resistance will lead to increased morbidity, mortality, and costs. Combining AQ with SP in those circumstances may not be useful; AQ with a sensitivity of 90% as monotherapy will be sufficient by itself and SP does not increase this sensitivity. To secure a longer life for AQ, it should be combined with an artemisinin drug, such as artesunate. In areas with less resistance, the combination of SP with AQ may secure a longer life for both drugs. In Kenya, comparative trials of SP plus AQ, AQ plus AS, and Coartem (Novartis, Basel, Switzerland) and preferably also dihydroartemisinin-piperaquine are needed. Further studies of several aspects of combination treatments such as use during pregnancy and infancy are urgently needed, but it is also time to act. Adequate funding is needed for these studies and for quick and large-scale implementation of their results.

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