INTRODUCTION

Malaria remains one of the most important infectious diseases worldwide. In sub-Saharan Africa, widespread resistance to chloroquine (CQ), the traditional first-line therapy for uncomplicated malaria, presents an important public health problem. Affected countries are faced with the challenge of selecting a new first-line regimen and revising antimalarial treatment policies.\(^1\) CQ is no longer effective in Central Africa.\(^2\)\(^-\)\(^5\) This situation is similar in the Central African Republic (CAR), where resistance of *Plasmodium falciparum* to CQ has been documented since 1983\(^6\) and resistance to sulfadoxine-pyrimethamine (SP) since 1987.\(^7\) Many other in vivo and in vitro studies have confirmed the slow progression of resistance to CQ in the CAR.\(^8\)\(^-\)\(^11\) The latest data in 1998 have shown that 48% of strains isolated in Bangui and less than 20% of strains in other towns in the CAR were resistant to CQ.\(^12\) No recent data exist concerning resistance to SP or amodiaquine (AQ).

Unfortunately, there is a limited choice of available and affordable antimalarials, and the optimal alternative to CQ is not clear. Current options for the treatment of acute uncomplicated chloroquine-resistant *P. falciparum* infections in Africa include the use of AQ or SP. The choice of these drugs is based not only on their clinical efficacy but also on their affordability to the great majority of African patients, good tolerance, safety for young children, and low toxicity risk. However, their high but not total clinical efficacy when used separately has been demonstrated in recent clinical trials conducted in chloroquine-resistant zones of Africa.\(^13\)\(^-\)\(^16\) Nevertheless, several African countries (not in the CAR) have adopted sulfadoxine-pyrimethamine (SP) as a replacement for CQ.\(^17\)

On the basis of field evidence from Southeast Asia, where combination therapy with artemisinin derivatives has maintained its efficacy and possibly delayed the spread of resistance,\(^18\)\(^,\)\(^19\) 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.

On the other hand, combination therapy has been advocated to improve efficacy and delay the development and spread of drug resistance.\(^20\) The use of SP with 4-aminoquinoline antimalarials offers the only available low-cost option for antimalarial combination therapy in Africa at this time. Some African countries, 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. Very few studies have compared the efficacy of multiple combinations without artemisinin derivatives in places with high CQ resistance and rapidly developing SP resistance.

Faced with high level of CQ resistance and to reevaluate its antimalarial treatment recommendations, the National Malaria Control Program (NMCP) in association with the Pasteur Institute of Bangui (IPB) have decided in a two-phase study in Bangui, CAR, first to reassess the clinical efficacy of CQ, the first-line antimalarial treatment, and second, to evaluate the therapeutic efficacy of AQ and SP, the available and affordable antimalarials in the CAR. Then, after the agreement of the Ministry of Health of the CAR and to confirm data from an unpublished study, conducted by NMCP in February to March 2003 based on the World Health Organization (WHO) standard protocol (WHO 2001), showing 100% clinical efficacy of AQ + SP combination in 62 enrolled children in Bangui, we undertook to evaluate the efficacy of two combinations: CQ + SP and AQ + SP.

METHODS

**Study area.** This study was carried out between February 2002 and March 2004 in three selected urban health centers of Bangui, the capital of the Central African Republic. The evaluation of the efficacy of CQ, SP, and AQ to update the first-line treatment was the first objective. This study was conducted between February 2002 to April 2002. Because of the high level of CQ, SP, and AQ resistance found in the first part of the study, it was decided with the agreement of the
NMCP to evaluate the efficacy of combinations CO + SP and AQ + SP, the only readily available and affordable drugs in the CAR. It was hardly possible to conduct this study before, because of conflict situations in the CAR in October 2002 and March 2003. So, this study was carried out between December 2003 to March 2004.

Bangui is located beside the Oubangui river in the heart of Central Africa in the north of the Democratic Republic of the Congo (geographic coordinates 7.00 N, 21.00 E). The climate is tropical and rainfall peaks from April to November. The average temperature varies from 19°C to 32°C. The duration of the malaria transmission is the entire year with peaks at the beginning and the end of the rain season. Malaria is hyperendemic, and the main parasite is *Plasmodium falciparum*. The parasite prevalence in children aged less than 5 years is 31.8%.21

**Patient recruitment.** Febrile patients aged 6–59 months presenting at the health facilities were enrolled in the study if we had informed consent from the parent or guardian and if they had an axillary temperature ≥ 37.5°C; monoinfection with *P. falciparum*, with parasitemia in the range of 2000–200,000 asexual parasites per microliter of blood; no other cause for fever than malaria; no general danger signs (unable to sit or stand up, unable to drink or breastfeed, lethargy or unconsciousness, recent history of convulsions, persistent vomiting) or signs of severe and complicated falciparum malaria according to the definition given by WHO (2001).22

To be included, participants should be able to come for the stipulated follow-up and needed access to the health facility. Parents or guardians were then interviewed by a study physician about symptoms, duration of the illness, previous antimalarial therapy, and other medications. Children were examined for pallor and jaundice or any other danger sign, and their axillary temperatures and weights were measured.

The lot assurance method (LQAS) according to WHO 1996 protocol23 was chosen to evaluate the sample size for each regimen.

**Treatments.** In the first part of the study, participants were treated with a standard dose of CO (Nivaquine; Aventis, Palaisseau, France), 10 mg/kg of body weight per day CO base for three days; SF (Fansidar; Roche, Rosny-sous-bois, France), 1.25 mg/kg body weight of pyrimethamine plus 25 mg/kg of body weight of sulfadoxine in a single oral dose; or AQ (Flavoquine; Aventis), 10 mg/kg of body weight per day for three days.

In the second part of the study, patients are treated with a combination of CO + SP or AQ + SP (same doses as in the other treatment groups given simultaneously on Day 0). Drugs were obtained from the UCM (Unité de cession des Médicaments) in Bangui. Doses were determined according to the modified weight-based guidelines from the WHO for administration of fractions of tablets. Patients from each site were alternately assigned to the different drug regimens. Personnel were not blinded to treatment. Drugs were administered by a medical doctor and repeated in case of vomiting within 30 min. In the case of a treatment failure or severe cases, quinine was given for 7 days. In addition, paracetamol was administered on Days 0 and 1.

**Patient follow-up.** Follow-up appointments were scheduled for Days 1, 2, 3, 7, and 14 and consisted of physical examination and completion of a standardized form. Parents and guardians were encouraged to return to the clinic at any time if their children felt unwell. Blood was collected by venous puncture on Day 0 (used to prepare thick and thin blood smears and evaluated hemoglobin and hematocrit levels) and by fingerprick on Days 3, 7, and 14 (used to prepare thick and thin blood smears). This was also performed whenever necessary based on clinical examination.

Patients who did not turn up on a scheduled day were visited at home. Patients were excluded from the study if 1) they reported self-administration of antimalarial drug during the follow-up; 2) they withdrew consent; 3) a concomitant disease occurred that would interfere with the clear classification of treatment outcome; 4) the patient moved out of the follow-up area. In any other condition, patients who did not turn up on a crucial day or were not seen at home were considered lost to follow-up.

**Laboratory tests.** Venous blood samples collected on Day 0 were used to prepare thick and thin blood films and evaluate hematocrit and hemoglobin levels and white blood cells count. The smears were stained with 4% Giemsa for 20 minutes. Parasite density was calculated by counting the number of asexual parasites per 200 white blood cells (WBC) and adjusting for the total WBC count. Thin blood smears were also examined for other *Plasmodium* spp. Hematocrit and hemoglobin levels and white blood cells count were determined by using an automated hematology analyzer (Coulter T540, Hialeah, FL).

**Outcome measures.** The clinical outcomes were classified into three categories of therapeutic responses. Early treatment failure (ETF) referred to development of danger signs or severe malaria on Days 1, 2, 3 or with parasitemia; axillary temperature ≥ 37.5°C on Day 2, with parasitemia higher than on Day 0; parasitemia on Day 3 with axillary temperature ≥ 37.5°C; parasitemia on day 3 ≥ 25% of count on Day 0. Late treatment failure (LTF) was defined as development of danger signs or severe malaria after Day 3 with parasitemia, without previously meeting any of the criteria for ETF; or parasitemia and axillary temperature ≥ 37.5°C on any day between Days 4 and 14, without previously meeting any of the criteria for ETF. Adequate clinical response (ACR) was constituted by absence of parasitemia on Day 14 irrespective of axillary temperature, without previously meeting any of the criteria for ETF or LTF; or axillary temperature < 37.5°C on Day 14 irrespective of parasite count, without previously meeting any of the criteria for ETF or LTF.

We also assessed parasitological failure (PF) by breaking down the results of the ACR group into two groups: ACR without parasitemia at Day 14 (adequate clinical and parasitological response; ACPR) and ACR with the presence of parasites at Day 14 but without fever (adequate clinical response and parasitological failure; ACR/PF).

**Statistical analysis.** Qualitative variables were compared by using either the $\chi^2$ test or Fisher’s exact test, and quantitative variables were compared by analysis of variance or the Kruskal-Wallis test. The 95% confidence intervals of percentages were calculated using the exact binomial test. The level of significance ($P$) was fixed at 0.05 for all statistical tests. The data were analyzed using the Epi Info program (version 6.04d).

**Ethics approval.** Because of lack of a national ethical committee in the CAR, this study was approved by the expert committee for the antimalarial drug policy in the CAR and the Ministry of Health in the CAR.
RESULTS

From February through April 2002 in the first study, 141 children were enrolled (23 in CQ group, 53 in AQ group, and 65 in SP group), of whom 8 (5.7%) were excluded (2 in CQ group, 3 in AQ group, and 3 in SP group), 4 (2.8%) lost to follow-up (1 in CQ group, 1 in AQ group, and 2 in SP group), yielding 129 (91.5%) who completed follow-up. Of these, 22 were allocated to the CQ group, 50 to the AQ group, and 57 to the SP group.

From December 2003 through March 2004 in the second study, 69 children were enrolled (46 in CQ + SP group, 23 in AQ + SP group), of whom 6 (8.6%) were excluded (4 in CQ + SP group, 2 in AQ + SP group), 3 (4.3%) lost to follow-up (2 in CQ + SP group, 1 in AQ + SP group), yielding 60 (87.1%) who completed follow-up. Of these, 42 were allocated to the CQ + SP group and 18 to the AQ + SP group.

The characteristics of the 189 patients followed up until Day 14 are summarized according to treatment group in Table 1. There was no significant difference (P > 0.05) in any of the parameters at baseline.

Therapeutic responses of CQ, AQ, CQ + SP, and AQ + SP groups are presented in Table 2. The overall treatment failure rate was 40.9% in the CQ group, 20.0% in the AQ group, 22.8% in the SP group, 7.2% in the CQ + SP group, and 0% in the AQ + SP group.

The fever clearance time (% of patients with apyrexia on Day 3) was significantly shorter in the CQ group (79%, 95% CI: 53.9–83.0), AQ (87.5%, 95% CI: 72.4–85.3), CQ + SP (87.5%, 95% CI: 88.3–99.9), and AQ + SP groups (94.2%, 95% CI: 84.0–100) than in the SP group (65.9%, 95% CI: 50.0–79.1) (P < 0.05).

The treatment regimens were well tolerated and no serious adverse effects were observed.

DISCUSSION

Our results in the first part of this study confirm those obtained by Bergeri and others in 1997–1998, suggesting that there is an urgent need for the CAR to withdraw CQ as first-line treatment and revise its antimalarial drug-use policy like many countries in Africa. We have enrolled only 16 children in CQ group, because our objective was to confirm that the CQ failure therapeutic rate exceed 25% and this drug could not be used as first-line treatment. One factor that may explain the high level of chloroquine resistance is drug pressure. Indeed, we have observed that in almost one-third of cases (37.5% ± 8.8%), chloroquine is the only antimalarial drug used in self-treatment and that it is often used at insufficient doses and stopped too early. Another possible explanation for the high level of CQ resistance is population movements: Bangui is the point where all the roads and rail links in the CAR converge, and the population of Bangui has grown rapidly in recent years because of immigration and a massive rural exodus. A large number of refugees arrived in the CAR from Rwanda in 1994 and from the Democratic Republic of the Congo in 1996, due to armed conflicts in these countries. These immigrants may have been carrying chloroquine-resistant strains because between 26% and 56% in 1985 and between 31.5% and 65.6% in 1985–1987 of P. falciparum isolates from these countries displayed CQ resistance, depending on the region. A similar phenomenon was described in a region of Zimbabwe, where the level of CQ resistance increased from 35% in 1984 to 83% in 1993 after the arrival of armed groups from Mozambique.

We also assessed the clinical efficacy of AQ and SP, the only readily available and affordable alternative antimalarial treatment in the CAR. No recent data exists about the efficacy of these drugs in the CAR. Like CQ, we found a high level of therapeutic failure, respectively 20% in AQ group and 22.8% in SP group. These results suggest that these drugs cannot be used alone as the first-line malaria treatment to replace CQ. Several studies have already shown a high level of AQ and SP resistance in Africa. 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 risk of selecting resistant parasites. However, it is striking to find such an AQ resistance level because AQ has not previously been intensely used in the CAR. Because it is a congener of CQ, cross-resistance may explain its low clinical efficacy.

In our study, combinations of SP with AQ or CQ were more effective than AQ or SP alone for treatment of uncomplicated malaria in Bangui. The CQ + SP combination had intermediate efficacy (92.8%). However, considering the

<table>
<thead>
<tr>
<th>Characteristic of patients</th>
<th>CQ</th>
<th>AQ</th>
<th>SP</th>
<th>AQ + SP</th>
<th>CQ + SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>22</td>
<td>50</td>
<td>57</td>
<td>18</td>
<td>42</td>
</tr>
<tr>
<td>Mean age (±SD), months</td>
<td>23.2 (±14.3)</td>
<td>24 (±12)</td>
<td>19.8 (±12)</td>
<td>22 (±15)</td>
<td>25 (±15)</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>0.84</td>
<td>0.94</td>
<td>1</td>
<td>2.4</td>
<td>1.21</td>
</tr>
<tr>
<td>Previous antimalarial therapy (%)*</td>
<td>35.3</td>
<td>34.8</td>
<td>34.6</td>
<td>52.9</td>
<td>30.0</td>
</tr>
<tr>
<td>Mean temperature (±SD) in °C</td>
<td>38.5 (±0.6)</td>
<td>38.5 (±0.6)</td>
<td>38.5 (±0.6)</td>
<td>38.3 (±0.6)</td>
<td>38.1 (±1.6)</td>
</tr>
<tr>
<td>Mean hematocrit (±SD), %</td>
<td>26 (±5)</td>
<td>22 (±6)</td>
<td>25 (±6)</td>
<td>29 (±7)</td>
<td>30 (±5)</td>
</tr>
<tr>
<td>Mean hemoglobin level (±SD), g/dL</td>
<td>8.3 (±1.8)</td>
<td>7.6 (±1.7)</td>
<td>7.9 (±1.7)</td>
<td>8.6 (±2.2)</td>
<td>9.1 (±1.6)</td>
</tr>
<tr>
<td>Mean parasitemia density (range) per μL</td>
<td>21,500 (2,000–86,500)</td>
<td>11,200 (2,000–64,500)</td>
<td>32,151 (2,000–88,044)</td>
<td>9,306 (2,400–40,258)</td>
<td>9,606 (2,080–34,740)</td>
</tr>
</tbody>
</table>

* Chloroquine was used in all cases.

<table>
<thead>
<tr>
<th>Therapeutic responses</th>
<th>CQ</th>
<th>AQ</th>
<th>SP</th>
<th>AQ + SP</th>
<th>CQ + SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>22</td>
<td>50</td>
<td>57</td>
<td>18</td>
<td>42</td>
</tr>
<tr>
<td>ETF</td>
<td>No. (%)</td>
<td>6 (27.3)</td>
<td>5 (10.0)</td>
<td>8 (14.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>LTF</td>
<td>No. (%)</td>
<td>3 (13.6)</td>
<td>5 (10.0)</td>
<td>5 (8.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>No. (%)</td>
<td>9 (40.9)</td>
<td>10 (20.0)</td>
<td>13 (22.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ACR</td>
<td>No. (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>ACPR</td>
<td>No. (%)</td>
<td>13 (59.1)</td>
<td>40 (80.0)</td>
<td>44 (77.2)</td>
<td>17 (94.4)</td>
</tr>
<tr>
<td>Total</td>
<td>No. (%)</td>
<td>13 (59.1)</td>
<td>40 (80.0)</td>
<td>44 (77.2)</td>
<td>18 (100)</td>
</tr>
</tbody>
</table>

ETF, early treatment failure; LTF, late treatment failure; OTF, overall treatment failure; ACR, adequate clinical response; PF, parasitological failure; ACPR, adequate clinical response and parasitological response.

fairly high prevalence of SP resistance demonstrated in this study and the very high prevalence of CQ resistance, it seems unlikely that the addition of CQ to SP will effectively protect from further selection of resistant parasites. The AQ + SP combination was significantly superior to the other regimens, providing excellent clinical efficacy and confirming data found in 2003 in a study conducted by the NMCP. This study was carried out between February 2003 and March 2003 in an urban health center in Bangui. Based on the WHO 2001 protocol, they assessed the clinical efficacy of AQ + SP combination in 62 enrolled children with uncomplicated falciparum malaria. At Day 14, adequate clinical responses was 100% (unpublished data). Several studies conducted in Rwanda, Uganda, and Cameroon on the AQ + SP combination have previously confirmed its excellent safety and efficacy. However, a limitation of our study was to have followed-up patients only for 14 days. Several studies have observed 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. These observations 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 the AQ + SP regimen is good, its long-term efficacy remains unknown. Indeed, because resistances to AQ and SP are rather high in our study, it is unlikely that the combination of both will efficiently protect against the development of further resistance.

Therefore, as recommended by the WHO, artemisinin-based combination therapies (ACTs) are probably the best antimalarial treatments.

However, other parameters than efficacy are to be considered, and the recommendation of AQ + SP as the interim first-line antimalarial treatment in the CAR has been established for three main reasons:

- First, the treatment must be affordable by the population. Indeed, when drug supply is controlled by the government, the cost of a complete curative dose of SP for an average adult weighing 60 kg is roughly equivalent to that of chloroquine (US$0.13), whereas AQ is nearly twice as expensive (US$0.20). The SP + AQ combination would thus cost three times as much as chloroquine or SP alone. In comparison, a 5-day course of treatment with quinine tablets costs more than nine times as much as chloroquine therapy. The SP + AQ combination is also much cheaper than halofantrine, artesunate, and co-artemether (10 to 20 times more expensive than chloroquine).
- Second, the association of AQ + SP has demonstrated its efficacy in the CAR at the moment and is better than the use of single drugs to prevent the development of resistance.
- Third, it seems to be safe, as no side effects were observed either in our study or in the study conducted by the NMCP in 2003.

In conclusion, these findings suggest that the Ministry of Health should recommend a interim policy with AQ + SP combination as the first-line antimalarial drug in Bangui, until best alternative treatments like ACTs become available at a low price in the CAR.

References


