EFFICACY OF SULFADOXINE/PYRIMETHAMINE IN THE TREATMENT OF UNCOMPROMICATED PLASMODIUM FALCIPARUM MALARIA IN REPUBLIC OF CONGO

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Abstract. Congo is facing frequent failures of treatment of Plasmodium falciparum malaria with chloroquine (CQ), which is still recommended and used as a first-line drug. In Pointe-Noire and Brazzaville, the two largest cities that contain approximately 60% of the population of Congo, we compared the efficacy of CQ versus sulfadoxine/pyrimethamine (SP) for treatment of uncomplicated malaria in children 6–59 months old (mean = 33 months) using the standard World Health Organization (WHO) 14-day in vivo test in two phases between 1999 and 2002. Patients enrolled were randomly assigned to receive SP (25 mg/kg of sulfadoxine and 1.25 mg/kg of pyrimethamine) or CQ (25 mg/kg). In the first phase of the study, 46 patients were assigned to the CQ (n = 23) or SP (n = 23) groups in Pointe-Noire and 52 children were assigned to the CQ (n = 26) or to SP (n = 26) groups in Brazzaville. Results were interpreted according to the WHO lot quality assurance sampling method, and treatment failure rates for SP versus CQ were < 25% versus > 25% in both cities. In the second phase of the study, we accurately determined the actual proportion of treatment failures for SP in Brazzaville. Thus, in 75 of the 80 children enrolled and followed-up until day 14, no clinical or parasitologic failure was recorded and no serious adverse reaction was observed. Since the CQ treatment failure rate exceeds the unacceptable upper limit, SP seems well to be an appropriate alternative for the first-line treatment of uncomplicated P. falciparum malaria, at least in the settings of the present study.

INTRODUCTION

Drug resistance in malaria continues to be a serious public health concern in Africa. Each year, an estimated 0.7–2.7 million people die of malaria, and more than 75% of them are African children. Rational policies for malaria therapy are the primary tools for responding to this re-emerging disease. The threat, development, spread, and intensification of chloroquine (CQ) resistance in sub-Saharan Africa are posing tremendous challenges to the national malaria control programs. If most countries south of the equator have already replaced CQ as the first-line therapy for uncomplicated malaria, Congo is still facing the complicated task of assessing the current status of drug resistance, making national policy-level decisions about whether or not to replace CQ, and initiating a monitoring system to track changes in the efficacy of malaria therapy. In Congo, malaria-attributable morbidity and mortality in children constitutes a major public health problem. The first report of CQ-resistant Plasmodium falciparum malaria in the country was in 1985. Since then, increasing reports of resistance of P. falciparum to CQ has created an urgent need for the study of an appropriate first-line therapeutic alternative. Most previous studies carried out throughout the country with the old World Health Organization (WHO) simplified seven-day in vivo test, principally in urban areas, had shown a critically high level of P. falciparum resistance to CQ. It has been reported that an increase in infant mortality was attributable to the escalating P. falciparum resistance to CQ. If the therapeutic efficacy is proved, the combination of sulfadoxine/pyrimethamine (SP), which is currently used as a second-line treatment in Congo, could be an ideal alternative first-line drug because of low cost, simple dosing, and relative safety. To assess the therapeutic efficacy of an antimalarial regimen, the relative proportion of treatment failures needs to be known. In addition, drug availability and use have been recognized as important determinants of drug resistance level. Several studies in neighboring countries reported a high therapeutic efficacy of SP. Sulfadoxine/pyrimethamine is now the first-line antimalarial drug in some African countries, such as Malawi, Kenya, Botswana, Tanzania, South Africa, and recently the Democratic Republic of Congo. The objective of this study was to assess whether CQ was still an acceptable first-line antimalarial drug and whether SP could be an alternative in case of high CQ treatment failures. Thus, we compared the efficacy of SP versus CQ in children 6–59 months old with uncomplicated P. falciparum malaria using the WHO 14-day in vivo test. This is the first such study to be carried out in the Republic of Congo.

PATIENTS AND METHODS

Study sites. Our study was carried out in two phases between 1999 and 2002. The phase I study (preliminary study) was designed as an open, randomized, controlled trial of two treatment regimens (CQ versus SP). Using the WHO Lot Quality Assurance Sampling (LQAS) method, this first study phase enabled us to know if the treatment failure rates to CQ and SP are lower or higher than 25%, the indicated threshold suggested by WHO for the replacement of the first-line drug. The phase II study was intended to determine the real proportion of treatment failures for each of these antimalarial drugs if the treatment failures rate was less than 25%.

This study took place in an urban area in Pointe-Noire (the economic capital) and in Brazzaville (the political and administrative capital), two cities that are 380 km apart in southern Congo. With 600,000 and 900,000 inhabitants, respectively, these two cities contain approximately 60% of the population of the country. As in the entire country, malaria is endemic and the transmission is perennial and peaks during
the rainy season, which normally occurs from October to May.

The phase I study was conducted in a central district of Pointe-Noire at Tié-Tié Hospital in the outpatient department of pediatrics between January and March 1999 and in a district of northern Brazzaville at Jane Vialle community health center between March and April 2001. The phase II study was conducted in a district of southern Brazzaville at Makélékélé Hospital in the outpatient department of pediatrics between February and April 2002.

Patients. The study was conducted in children with fever aged less than five years old who were attending a health center. Using the WHO 14-day in vivo protocol, patients were enrolled in the study if they satisfied the following inclusion criteria: age between 6 and 59 months, monoinfection with P. falciparum, parasitemia ≥ 2,000 asexual parasites per microliter of blood, free from severe malnutrition, absence of general danger signs (i.e., inability to stand, breastfeed, or drink, recent convulsions, lethargy, or persistence vomiting), severe and complicated malaria, an axillary temperature ≥ 37.5°C, absence of febrile conditions caused by diseases other than malaria, ability to come for the stipulated follow-up visits and easy access to the health facility, informed consent of parents or guardians, absence of history of hypersensitivity reactions to sulfonamides and a hemoglobin level of at least 5 g/dL.11 Neither a history of previous antimalarial drug use nor the presence of antimalarial drugs in the urine was an exclusion criterion in following with the 1996 WHO protocol.11 Before enrolment in the study, a medical history of each patient was obtained from their accompanying parent or guardian and the child was clinically examined by a physician. Body weight and axillary or ear temperature were recorded and thick and thin blood smears were stained with 5% Giemsa for 20 minutes for parasite identification and quantification. Parasitemia (parasites/microliter) was measured by counting the number of asexual parasites per 200 leukocytes in the Giemsa-stained thick blood smears, basing on a mean count of 8,000 leukocytes per microliter of blood. A slide was declared negative only after microscopic fields corresponding to at least 500 leukocytes had been checked. Two experienced technicians performed the microscopic analysis independently, each time comparing their results. The principal investigator closely supervised the study team to ensure consistency and accuracy of the data. Some slides randomly chosen were re-read in Paris.

The hemoglobin level was measured on day 0 using the Tallquist method.18 The Säker-Solomons test was used to detect CQ and its metabolites in urine specimens from patients randomly selected in the target population to estimate the drug pressure level.19

In the phase I study, sample size estimations for the in vivo tests were performed using the WHO LOQAS method.11 We assumed that the proportion of treatment failures for CQ in the urban area population of patients with uncomplicated P. falciparum malaria was higher than 25% (research hypothesis); this threshold was considered according to WHO criteria as an indication for first-line drug policy change.11 Thus, by the LOQAS method, the number of treatment failures greater than five in an initial sample size of 16 patients was sufficient to assert (95% confidence level, 80% power) that the proportion of clinical failures was not significantly less than 25% in this population. In the phase II study, the sample size was calculated with the same method, assuming that the treatment failure rate would be significantly less than 15% for SP because the phase I study showed a treatment failures rate less than 25% for SP and greater than this threshold for CQ. This resulted in a minimum required sample size of 50 patients.

Treatment and follow-up. Phase I study. After informed consent was obtained from parents or guardians on day 0, the enrolled children were randomly allocated (using a table of random numbers) to one of two treatment groups to receive either CQ base (chloroquine tablets; CHMP Clermont-Ferrand, France), 25 mg/kg of body weight over a three-day period (i.e., 10 mg/kg, 10 mg/kg, and 5 mg/kg), or SP (Fansidar® tablets; Roche, Paris, France) at a single dose of 25 mg/kg of sulfadoxine and 1.25 mg/kg of pyrimethamine.

Phase II study. Based on our study design, only the therapeutic efficacy of SP was assessed in this phase for determining the actual proportion of treatment failures with the same treatment modality.

An antipyretic (paracetamol, 15mg/kg, every 8 hours for 24 hours) was systematically given on day 0 and if needed on days 1 and 2. All tablets were administered orally by a nurse in the presence of the physician. For 30 minutes following drug administration, patients were observed for vomiting and other side effects. The same dose was re-administered if vomiting occurred. On days 1 and 2, symptoms, other medications, temperature, and physical examination were recorded, but microscopy was not performed unless one or more of danger signs were present. The same clinical observation was repeated and a parasitologic examination was conducted on days 3, 7, and 14. On day 0, when the patient presented a fever without parasitemia in the absence of another pathology, the child was seen the next day for intensive follow-up and microscopic diagnosis of malaria. All treatment failures were treated with quinine tablets (8 mg/kg base three times a day for seven days). The responses to drug treatment were classified as early treatment failure (ETF), late treatment failure (LTF), and adequate clinical and parasitologic response (ACPR).11 The drug treatment is considered as an ETF if the patient develops danger signs or severe malaria on days 1, 2, or 3 in the presence of parasitemia, presented with a parasitemia on day 3 higher than that on day 0 irrespective of the axillary temperature, presented with a parasitemia on day 3 with an axillary temperature ≥ 37.5°C, or presented with a parasitemia on day 3 ≥ 25% of count on day 0. The response to treatment is classified as LTF, which includes late clinical failure (LCF) or late parasitologic failure (LPF), if the patient develops 1) danger signs or severe malaria in the presence of parasitemia on any day from day 4 to day 14 without previously meeting any of the criteria of early treatment failure, an axillary temperature ≥ 37.5°C in the presence of parasitemia on any day from day 4 to day 14 without previously meeting any of criteria of early treatment failure (LCF), and 2) the presence of parasitemia on day 14 and an axillary temperature < 37.5°C without previously meeting any of criteria of early treatment failure or late clinical failure (LPF). Adequate clinical and parasitologic response is an absence of parasitemia on day 14 irrespective of the axillary temperature without previously meeting any of the criteria of ETF or LTF.

Ethical considerations. The local health and institutional authorities (Ministry of Public Health) reviewed and approved the research protocol. Verbal and written informed consent for participation were obtained from parents or
Clinical and biologic parameters at enrollment of the patients assigned to treatment with chloroquine (CQ) or sulfadoxine/pyrimethamine (SP) compared by calculating chi-square values with Yates (Graph Pad Software, San Diego CA). Proportions were provided in the local language.

Statistical analysis. Data were analyzed using version 2000 of the Epi-Info software (Centers for Disease Control and Prevention, Atlanta, GA) and Graphpad Prism software (Graph Pad Software, San Diego CA). Proportions were compared by calculating chi-square values with Yates’ correction or preferably Fisher’s exact test. Normally distributed, continuous data were compared using Student’s t-tests and analysis of variance. Data not conforming to a normal distribution were compared using Mann-Whitney U tests and Kruskal-Wallis tests. Confidence intervals of 95% were used.

The standard deviation was generally indicated for means and P values < 0.05 were calculated to demonstrate statistical differences.

RESULTS

Clinical features and therapeutic responses in the phase I study. Overall, 46 children were enrolled in Pointe-Noire and 52 children were enrolled in Brazzaville. The patients came from all districts in both cities. The study populations were similar at both sites and no difference was observed in the therapeutic groups in terms of sex, mean age, mean body weight, parasitemia, and geometric mean levels of hemoglobin. No patient was excluded because of severe anemia. The clinical and biologic features of the study population at enrollment at both sites are summarized in Table 1.

Of the 46 enrolled children in Pointe-Noire, 23 were randomly placed in the CQ group and the other 23 in the SP group. Four patients were excluded or lost to follow-up (two in CQ group and two in SP group) during the follow-up period because of failure to follow the protocol (antimalarial treatment administered by themselves or a third party) or failure to come for follow-up on the scheduled days (generally because of travel for several days outside the city). Seven treatment failures were observed in the first 16 patients in the CQ group and no treatment failures were observed in the first 16 patients in the SP group. This indicated, based on the WHO LQAS method, that the treatment failure rate in the study population was higher than 25% for CQ and less than 25% for SP. However, other patients were already enrolled in addition to the first 16 and we followed them until day 14. Overall, treatment failures were significantly higher in the CQ group (9 of 21, 42.8%) than in the SP group (2 of 21, 9.5%; P < 0.05); patients lost to follow-up were excluded from the analysis.

Results similar to those in Pointe-Noire were observed in Brazzaville where 52 children were enrolled, 26 of whom were treated with CQ and the other 26 with SP. Two patients were lost to follow-up or excluded in each therapeutic group (because of the aforementioned reasons). The treatment failure rate was greater than 25% in CQ group and less than 25% in the SP group. Other patients in addition to the first 16 were followed until day 14. Overall, treatment failures after 14 days were significantly more frequent in the CQ group (15 of 24, 62.5%) compared with the SP group (1 of 24, 4.2%; P < 0.05); patients lost to follow-up or excluded were withdrawn from the analysis. Overall, no severe adverse drug event was observed during follow-up of the patients in any treatment group at either site. The clinical and parasitologic responses of the phase I study are presented in Table 2.

Urinary specimens collected from randomly selected children in the target population (children with fever attending a health center) were tested to identify CQ and its metabolites. The urine Saker-Solomons test result was positive in 56% (28 of 50) in Pointe-Noire and 52.8% (28 of 53) in Brazzaville, indicating frequent previous intake of CQ (the difference was not statistically significant in either study area; P > 0.05).

Therapeutic responses in the phase II study: determination of the actual proportion of treatment failures for SP. Since the treatment failure rate was less than 25% for SP in the phase I study, this second phase sought to determine more accurately the actual rate of treatment failures. Eighty outpatients 6–59 months old who came from all districts of Brazzaville were recruited. Two patients were lost to follow-up and three others were excluded during the follow-up period.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase I study</th>
<th>Phase II study</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pointe-Noire</td>
<td>Brazzaville</td>
</tr>
<tr>
<td></td>
<td>CQ (n = 23)</td>
<td>CQ (n = 26)</td>
</tr>
<tr>
<td></td>
<td>SP (n = 23)</td>
<td>SP (n = 26)</td>
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<tr>
<td>Age (months)</td>
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<tr>
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<tr>
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<td>Weight (kg)</td>
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<td>Range</td>
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<td>Temperature (°C)</td>
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<td>Mean (SD)</td>
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<td>Hemoglobin (g/dL)</td>
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<td>9.5 (1.2)</td>
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<tr>
<td>Mean (SD)</td>
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<tr>
<td>Range</td>
<td>9.6 (1.3)</td>
<td>9.7 (0.7)</td>
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</tbody>
</table>

* Comparison of all means.
Clinical and parasitologic outcomes of the phase I study in Republic of Congo, 1999–2001*

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Brazzaville (CQ, n = 26)</th>
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<td>Adequate clinical and parasitologic response</td>
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<td>19 (90.5)</td>
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<td>Early treatment failure</td>
<td>4 (19.0)</td>
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<tr>
<td>Late treatment failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late clinical treatment failure</td>
<td>4 (19.0)</td>
<td>2 (9.5)</td>
<td>0.662</td>
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<tr>
<td>Late parasitologic failure</td>
<td>1 (4.8)</td>
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<td></td>
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<tr>
<td>Patients lost to follow up or excluded†</td>
<td>2 (8.7)</td>
<td>2 (8.7)</td>
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</table>

* CQ = chloroquine; SP = sulfadoxine/pyrimethamine.
† Excluded from the analysis.

because of the same reasons already stated in the phase I study. The clinical and biologic parameters at enrollment did not differ from those of the phase I study (P > 0.05 for all comparisons, Table 1). The hemoglobin test was not undertaken during phase II. The clinical and biologic features at enrollment of the patients are summarized in Table 1.

Patients excluded and lost to follow-up were withdrawn from the analysis. Among the 75 patients who were followed-up until day 14, no clinical or parasitologic failure was observed, and no serious adverse reaction was recorded (one patient reported pruritus on day 1). Most patients (93.3% [70 of 75]) had a normal temperature by day 2, and parasite clearance was obtained in 74 (98.7%) of 75 patients by day 3. The therapeutic efficacy outcomes of the phase II study are shown in Table 3. The quality control of blood films from each site conducted by the National Malaria Reference Center in Paris, France showed a degree of disagreement less than 3%.

**DISCUSSION**

Chloroquine resistance is alarmingly high in Congo, and there is an urgent need for a cheap, effective, safe, readily available, and affordable antimalarial drug as an alternative at the first-line treatment level for uncomplicated malaria. In this country, this is the first study using clinical and parasitologic criteria according to the WHO 14-day *in vivo* test. The CQ and SP used in this study were provided by authentic French firms and the quality control of blood films was carefully done.

This study was particularly unique in several important aspects. First, the study was carried out during the period when Congo suffered civil wars from 1993 to 2002. This situation caused perpetual movements of the urban populations from one district to another (change of residence) and a profound modification of the urban structure, particularly in Brazzaville. Second, in each study site, the patients came from all districts of the city. This combination of circumstances is an important argument to extrapolate the research findings to the entire urban region.

Our results demonstrated, both clinically and parasitologically, a statistically significant better efficacy of SP compared with CQ, for which resistance has reached unacceptable levels with a treatment failure rate greater than 25%, prompting a policy change according to WHO. Both regimens were well tolerated, with no serious adverse reactions recorded during the course of the study. Most (93.3%) patients were afebrile by day 2, and parasite clearance was observed in nearly all (98.7%) patients by day 3. The Saker-Solomons urine test result was positive in 56% in Pointe-Noire and in 52.8% in Brazzaville, indicating intense drug pressure in the urban area, especially with CQ. This increasing drug pressure of CQ has already been observed in more than 50% of the cases in Brazzaville since 1987. Different observations might explain this high level of drug pressure for CQ.

Despite alarming resistance to CQ, this antimalarial drug still continues to be used as “a miracle drug” by the population because other alternatives (e.g., mefloquine) are much more expensive, difficult to administer (e.g., quinine for seven days), or not encouraged (e.g., SP and amodiaquine). However, the greatest deterrent is the lack of a rational malaria-therapy policy at the national level in Congo. The national malaria-therapy policy would describe antimalarial drugs available for use, their relative efficacy, and how best to use them in a variety of settings, from the community to the referral hospital. Several studies had confirmed that the most important determinant of increased drug resistance appears to be increasing drug pressure, especially when combined with intense transmission. Previous studies were conducted in Congo, particularly in urban areas, with the old WHO 7-day *in vivo* test and all reported alarming parasito-
logic resistance rates.3–6 Although the methodology is different from our own, we believe that resistance to CQ is present in urban areas of Congo. Similar data from neighboring countries show that they are also facing high levels of CQ resistance.15–21

With regard to the effectiveness of SP, the only previous study carried out in Brazzaville in 1988–1989 in 40–3–14-year-old outpatients with malaria showed no clinical resistance after a seven-day follow up, and low persistent parasitemia was seen in two cases.4 There is strong evidence that SP is still an efficacious drug in these urban settings. The therapeutic efficacy and the tolerance of SP in the treatment of uncomplicated P. falciparum malaria was also reported in studies carried out in neighboring countries. Data reported from Cameroon showed an adequate ACPR rate of 100% on day 14 and 84.7% on day 28.12 In Gabon14 and the Democratic Republic of Congo,15 the therapeutic efficacy rates of SP reported were 98% and 87%, respectively, Beyond the frontiers of central Africa, SP was also judged to be effective. Some of countries of east Africa have already made the decision to change from CQ to SP for first-line treatment of uncomplicated malaria.17,24–26

The main limitation of this study is that follow-up period was not extended beyond 14 days. The standard 14-day follow-up period recommended by WHO for areas of intense transmission, and used in this study, might not have been sufficient to detect late emergence of resistant parasites, and thus, some resistant outcomes could have been missed. However, the 14-day follow-up restricted the misclassification of new infections presenting more than two weeks after therapy as treatment failures.27 In addition, for rapidly eliminated drugs, a 28-day follow-up is needed, but, for slowly eliminated drugs, up to nine weeks could be required to document all recrudescences. When possible, molecular genotyping to distinguish therapeutic failures from reinfections and drug dosages should also be undertaken, but this is expensive.28 In trials carried out in areas where SP still remains effective, we suggest that after a 14-day follow-up, a smaller sample of patients in the therapeutic groups be randomly chosen according to WHO LQAS method to estimate the true risk of resistance beyond 14 days, rather than following-up the entire cohort. This strategy would limit the costs of the studies and could simplify public health decisions. In contrast, other investigators reported that when monitoring the efficacy of commonly used antimalarial drugs over a long period, extending the follow-up beyond 14 days might not be required.25

In conclusion, since the level of CQ treatment failures exceeds the unacceptable upper limit, SP seems to be an appropriate alternative for the first-line treatment of uncomplicated P. falciparum malaria, at least in the settings of the present study. However, other studies should be carried out in diverse geographic settings throughout Congo so that useful decisions and changes in the national drug policy can be made. The study of SP in combination with other effective, cheap, safe, and affordable antimalarial drugs in delaying the development of parasite resistance is essential.

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