Comparison of Different Artemisinin-based Combinations for the Treatment of *Plasmodium falciparum* Malaria in Children in Kigali, Rwanda, an Area of Resistance to Sulfadoxine-Pyrimethamine: Artesunate Plus Sulfadoxine/Pyrimethamine versus Artesunate Plus Sulfamethoxypyrazine/Pyrimethamine

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Abstract. In view of the changing policy towards artemisinin-based combination therapies (ACTs), the efficacy, tolerance, and degree of re-infection of two ACTs were investigated: artemesunate plus sulfadoxine/pyrimethamine (As + SP) and AS plus sulfamethoxypyrazine/pyrimethamine (As + SMP). One hundred three children were assigned to receive As + SP and 109 to receive As + SMP. In spite of the high incidence of resistance to SP, As + SP showed satisfactory results consistent with recent recommendations for ACTs (adequate clinical and parasitologic response on day 28 [ACPR] ≥ 90%), but results with As + SMP fulfilled the most stringent criteria (ACPR ≥ 95%). The absence of side effects and the low price of these drugs make them it worth to reconsider national therapies in favor of either of these two drug combinations.

INTRODUCTION

Malaria is one of the most important infectious diseases worldwide. In Africa, which bears the greatest burden of this disease, controlling efforts have been largely unsuccessful. New therapies are urgently needed and it is generally agreed that an artemisinin-based combination therapy (ACT) offers the best opportunity for effective treatment and prevention of selection of drug-resistant parasites. The two most recommended ACTs in Rwanda are the combinations of artesunate and amodiaquine (As + AQ) and arteether and lumefantrine (AL). However, because of the emergence of resistance to and the degree of re-infection with these drugs, the value of these combinations has been questioned.

In a study by Rwagacondo and others, the safety and efficacy of As + AQ in treating uncomplicated *Plasmodium falciparum* malaria was investigated. They concluded that As + AQ increases the efficacy of treatment. However, the apparent increase of resistance to AQ observed in only a one-year period is of concern and casts doubts on the suitability of implementing As + AQ as first-line treatment in Rwanda. They recommend that alternative treatments should be identified and tested. Coartem® (Novartis, Basel, Switzerland), a fixed-dose combination of AL, was considered a possible alternative treatment, and a randomized, open-label, clinical trial to test its safety, tolerability, and efficacy was carried out by Fanello and others. **Artemether and lumefantrine was shown to be efficacious with a cure rate of more than 95%, with a good safety and tolerability profile. However, in areas with high malaria transmission and drug resistance, this combination was less effective. Mutabingwa and others observed a high re-infection rate of approximately 20% after treatment with AL in the high transmission and drug-resistance area of northern Tanzania in 2005.** Similar results were obtained in 2006 with a study in five sentinel sites in Zambia conducted by the National Malaria Control Center. They found re-infection rates between 19.2% and 53.8% for AL.

On the basis of the published reports and consistent with recent recommendations of the World Health Organization (WHO), certain drug combinations may be inferior to other ACTs. It has been recommended that the combination of As with sulfadoxine/pyrimethamine (SP) should not be used in areas where resistance to SP exceeds 20%. In western Africa, resistance is not widespread; therefore, the probability for successful treatment is increased. However, conflicting data have been reported. For example, in Ghana the resistance rate to SP is 35%, but As + SP shows a recrudescence rate of only 5% after correction by polymerase chain reaction (PCR). Reports by WHO showed that in areas with a high estimated rate of resistance to SP, the recrudescence rate with As + SP was 26% in Kenya and 25.6% in Uganda. In a study in Rwanda, in which recrudescence rates to SP were found to be as high as 44.2% in certain areas, PCR-corrected recrudescence rates after treatment with As + SP ranged from 5.6% to 17.9%. Another study in Gabon that tested a three-day course of AS (4 mg/kg of body weight) showed a cure rate of 72%. In contrast, studies in Angola, Zambia, and Sudan showed the most encouraging results with a combination therapy of As + SP with recrudescence rates of only 1.2%, 2%, 0.9%, 0%, and 0.7% respectively, in spite of a variable but rather high level of resistance to SP.

In a recent randomized controlled study in Bamako, Mali, a combination of AS with sulfamethoxypyrazine/pyrimethamine (SMP), an alternative long-acting sulfonamide, gave a cure rate of nearly 100%, whereas AL showed inferior results for the recrudescence and reinfection. Sulfamethoxypyrazine/pyrimethamine has a long but stable elimination half-life of approximately 80 hours, and its low plasma-binding capacity (65%) enables use of a low dosage with a long-lasting effect on parasites.

We therefore assessed treatment with As + SP compared with As + SMP in an area with a high level of resistance to SP. Our study had two objectives. The first objective was to assess treatment with As + SP in an area with a high level of resistance to SP. The second objective was to evaluate differences in effectiveness between two sulfa drugs (sulfadoxine and sulfamethoxypyrazine).

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SUBJECTS AND METHODS

Study design and rationale. This was an open-label randomized clinical trial comparing the efficacy of two sulfa drugs with pyrimethamine in combination with As for antimalarial treatment of children. The patients were randomly assigned to one of the two study regimens: As + SP (Ariplus®; Dafra Pharma NV, Turnhout, Belgium) or As + SMP (Co-Arinate®; Dafra Pharma NV). We studied these two co-blister combination therapies to assess tolerability and safety of both regimens and to evaluate which is more effective in the treatment of uncomplicated malaria in Rwandese children. The study protocol was reviewed and approved by both the Rwandan National Ethical Committee and the Research Commission of Central University Hospital of Kigali.

Study center and patients. The trial was conducted in Kigali, Rwanda. All patients recruited for this trial were residents of Kigali for the complete duration of this trial and attended one of the health centers participating in this trial. They were recruited at three different locations (Muhima, Gikondo and Kicukiro), as shown in Figure 1.

Children three months to twelve years of age who weighed more than 5 kg and attended one of the three health centers were considered eligible for enrollment provided they met all inclusion and exclusion criteria. They were screened over a period of 17 weeks (September 2005–January 2006) and were included in the trial if they were infected with P. falciparum, if they had a history of fever over the preceding 24 hours, and if they had not received any anti-malarial medication in the preceding two weeks. Only children from whom a signed informed consent was obtained from their parent or legal guardian were admitted into the study. Exclusion criteria were an inability to take oral medication, signs of severe malaria, a history (obtained from the parent or guardian) of any known serious chronic disease that required frequent medical care (e.g., acquired immunodeficiency syndrome, sickle cell disease, malignancy), and a history (obtained from the parent or guardian) of serious side effects to study medications.

Drug regimens. The children were randomly assigned to one of the two treatment regimens and received their medication over a period of 48 hours. After admission into the study, they received tablets at 0, 24, and 48 hours. Patients in the As + SP group received As (4 mg/kg/day) plus SP (40 mg/kg of sulfadoxine and 2 mg/kg of pyrimethamine). They received the As tablets in three doses over 48 hours and the SP tablets as one dose at 0 hours. Patients in the As + SMP group received As (4 mg/kg/day) plus SMP (10 mg/kg/day of SM and 0.5 mg/kg/day of pyrimethamine). They received both types of tablets as a single combined dose once a day for three days every 24 hours. All patients received the medication under supervision of a qualified person. In case of vomiting within 30 minutes after intake of the medication, the full dose was repeated. If this occurred between 30 and 60 minutes after administration, half the dose was re-administered. Patients who could not retain their medication for a second time were excluded from the trial and were provided an alternative treatment according to hospital policy.

General study procedures. Upon recruitment, all patients received a three day-treatment (days 0, 1, and 2). They were asked to return to the hospital on days 3, 7, 14, 21, and 28 for follow-up to monitor the treatment outcome. At each consultation, a general physical examination was performed to determine fever clearance and elimination of parasites.

Peripheral blood samples obtained by finger prick were collected at each consultation for generation of thick blood films (slides) and for analysis by PCR (filter papers) in cases of treatment failure. After thick blood films were stained with Giemsa, each slide was read by two microscopists independently. In case of any discrepancy, a third reviewer would read the slides.

Hemoglobin levels were also monitored on days 0 and 28 for evaluation of drug effects on hemoglobin content. Drug-related adverse events were recorded at each consultation. Patients were encouraged to return to the health centers at any time they felt sick on a day that was not included as a follow-up day in the protocol.

Definition of endpoints. Classification of the treatment outcome was based on an adequate clinical and parasitologic response on day 28 (ACPR) for an absence of PCR-corrected parasitemia on day 28. Patients who had danger signs or severe malaria during the first three days in the presence of parasitemia were classified as early treatment failures. Patients who had recurrent fever and parasitemia between days 3 and 28 were classified as late clinical treatment failures. Patients who had recurrent parasitemia without fever between days 7 and 28 were classified as late parasitologic treatment failures.

Adjustment for reinfection. In areas of high transmission of malaria, patients are often reinfected. This reinfection confounds treatment outcomes. To assess a correct conclusion regarding appearance of treatment failure, a clear distinction had to be made between cases of reinfection and recrudescence for patients with recurrent parasitemia after day 7. Blood samples collected during the trial (on day 0 and the day of parasitemia recurrence), were sent to the Malaria Research and Training Centre (University of Bamako, Bamako, Mali) for analysis of parasite merozoite surface proteins and glutamate-rich protein. Recrudescence was distinguished from reinfection by parasite genotyping using a PCR method.18

Statistical design and data analysis. This trial was a pilot study; thus, conclusions are considered exploratory. A total of
212 patients were recruited and randomly divided into the two treatment arms. The chosen null hypothesis was that the treatment efficacy of As + SP equaled that of As + SMP. The clinical and parasitologic efficacy of both treatments was compared using Fisher’s exact test. Fever clearance and parasitologic clearance were compared by a log rank test. Fisher’s exact test was also used to evaluate the proportion of undesirable effects between the two treatment arms. Data was analyzed using Stata version 9 (Stata Corporation College Station TX) and SPSS version 12.0 (SPSS Inc., Chicago, IL).

RESULTS

A total of 4,170 children with fever were screened at the three sites. Of these children 3,958 were excluded because they did not meet the inclusion criteria; 212 were included in the trial. Baseline characteristic of the enrolled patients are shown in Table 1. Of the 212 patients, 103 (48.58%) received As + SP and 109 (51.42%) received As + SMP (Figure 2). All patients received their medication on the foreseen timepoints (days 0, 1, and 2) and returned for monitoring as scheduled on days 3, 7, 14, 21, and 28. Eight of the patients were lost to follow-up (5 in As + SP group and 3 in As + SMP group) because they moved from the study area and were not retraceable, and 2 others were excluded because of protocol violations (taking supplementary medication at home). These 10 patients were replaced with new patients. The baseline characteristics of these new patients showed no statistical difference with those who were excluded or lost to follow-up (Table 2).

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**Table 1**

Baseline characteristics of enrolled patients*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Kicukiro</th>
<th>Gikondo</th>
<th>Muhima</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients recruited</td>
<td>85 (40.09%)</td>
<td>32 (15.09%)</td>
<td>95 (44.81%)</td>
<td>212 (100%)</td>
</tr>
<tr>
<td>Mean age in months (95% CI)</td>
<td>57.7 (49.6–65.9)</td>
<td>68.4 (52.0–84.7)</td>
<td>61.4 (52.7–70.1)</td>
<td>61.0 (55.4–66.6)</td>
</tr>
<tr>
<td>Sex ratio F/M</td>
<td>36/49 = 0.73</td>
<td>12/20 = 0.6</td>
<td>45/50 = 0.9</td>
<td>93/119 = 0.78</td>
</tr>
<tr>
<td>Mean axillary temperature on presentation, °C (95% CI)</td>
<td>38.0 (37.8–38.2)</td>
<td>37.7 (37.4–38.0)</td>
<td>37.9 (37.7–38.1)</td>
<td>37.9 (37.8–38.0)</td>
</tr>
<tr>
<td>Mean asexual parasitemia per microliter of blood on presentation (95% CI)</td>
<td>34,729.13 (26,522.7–42,935.56)</td>
<td>6,556.41 (4,034.81–9,078.01)</td>
<td>40,768.19 (22,491.54–59,044.84)</td>
<td>33,182.83 (24,237.98–42,127.68)</td>
</tr>
</tbody>
</table>

*CI = confidence interval.

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*Figure 2. Profile of the study. AS+SP = artesunate plus sulfadoxine/pyrimethamine; AS+SMP = artesunate plus sulfamethoxypyrazine/pyrimethamine.
Clinical and parasitologic efficacy. None of the patients in both treatment arms showed early treatment failure during the first three days after initiation of treatment. However, after day 7, some patients again had a parasitemia after initial clearance of the parasites. Eighteen patients who received As + SP showed late treatment failure and 11 patients who received As + SMP showed late treatment failure. After correction by PCR, 10 (9.71%) patients in the As + SP group showed recrudescence and 4 (3.67%) patients in the As + SMP group showed recrudescence. The rest of the treatment failures were caused by a new infection: 8 (7.77%) in the As + SP group and 7 (6.42%) in the As + SMP group. This resulted in an efficacy of 90.3% for As + SP and 96.3% for As + SMP (odds ratio = 0.3543, 95% confidence interval [CI] = 0.10624–1.18145, P = 0.0775). Thus, the null hypothesis was not rejected and there was no significant difference in efficacy between the two drugs.

Hemoglobin levels were also monitored on days 0 and 28 to evaluate drug effects on hemoglobin. The results are shown in Table 3 by age categories. Mean total hemoglobin levels on day 28 (mean = 10.07; 95% CI = [9.84–10.3]) were higher than those on day 0 (mean = 9.72; 95% CI = [9.49–9.95]). There was no report of serious adverse events and none of the patients followed-up reported any drug-related side effects. Some mild adverse events such as weakness were noted, but these events resolved spontaneously and gave no reason to stop the treatment. Thus, both drugs were effective in treatment of uncomplicated malaria in children. The difference in recrudescence indicates that AS + SMP may be the preferred treatment.

DiscUSSION

Artesunate-based combination therapy is now becoming the only acceptable tool for treating non-complicated malaria. However, there are several options and it is not always easy to choose which drugs might be best suited for a specific population. Although WHO strongly favors use of AL (Coartem®), other combinations need to be considered. Regarding use of AS + AQ, it is known that AQ causes unpleasant side effects in some patients.19 Therefore, patients tend to refuse to take the yellow AQ pills. Artemether-lumefantrine is expensive, even at a discount,20 and costs approximately $9–$12 per treatment. Combinations such as AS-mefloquine are not commonly used in Africa. Mefloquine can cause frequent side effects,21 and its cost is also rather high. Combinations with other antimalarial drugs such as piperaquine or pyronaridine are not used frequently enough to properly evaluate them. Thus, a careful reconsideration of the low-cost combination with SP was necessary, particularly in the context of some published data that suggested that this combination might be lacking efficacy. Published data6–9 stimulated this reassessment.

Resistance to SP is considered to be high in Rwanda, but accurate data are missing. It is believed that today resistance exceeds 50%. If the assumption is correct that a combination of As with a longer-acting drug against which resistance is high should not be used, then all As + SP combinations would not be used. We therefore re-examined this suggestion. Because positive results were reported with an alternative sulfonamide combination,16 we compare two drugs.

Sulfamethoxypyrazine has theoretical advantages over sulfadoxine. Apart from being a safe drug, sulfamethoxypyrazine is less bound by protein in plasma than sulfadoxine and a smaller dose will have a longer effect.22 Both preparations are available in co blister forms, but the dose is slightly different. With As + SP, sulfonamide is given as a single dose on day 0, whereas in the combination with sulfamethoxypyrazine, As and the sulfonamide are taken together with a 24-hour interval. The tolerance of both drugs was excellent and drug-related side effects were not observed. The outcome ACPR was more favorable for the combination with sulfamethoxypyrazine (96.3%) than with sulfadoxine (90.3%, P = 0.0775), but because both drugs have ACPRs greater than 90%, both combinations are useful.

These findings contrast strongly with data published in other studies conducted in the same subregion. Obonyo and others showed treatment failure in 26% of cases in a Kenyan study,7 and Priotto and others confirmed this finding and reported a failure rate of 25.6% in a similar study in Uganda.8 It was expected that in the current study rather unfavorable results might be expected in view of published data. Our study showed that the combinations with As are useful and slightly

<table>
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<th>Table 2</th>
<th>Baseline characteristics of the 10 subjects who were replaced by new patients</th>
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<tbody>
<tr>
<td>Patients</td>
<td>Standard</td>
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<tr>
<td>Mean</td>
<td>Mean</td>
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<tr>
<td>Temperature, °C</td>
<td>Abandoned 37.35</td>
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<tr>
<td></td>
<td>Replaced 37.75</td>
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<tr>
<td></td>
<td>Difference 0.4</td>
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<tr>
<td>Hemoglobin level, g/dL</td>
<td>Abandoned 11.52</td>
</tr>
<tr>
<td></td>
<td>Replaced 11.28</td>
</tr>
<tr>
<td></td>
<td>Difference −0.24</td>
</tr>
<tr>
<td>Parasitemia, parasites/µL</td>
<td>Abandoned 30,134</td>
</tr>
<tr>
<td></td>
<td>Replaced 30,802</td>
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<tr>
<td></td>
<td>Difference 52</td>
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<table>
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<tr>
<th>Table 3</th>
<th>Hemoglobin data by age categories*</th>
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<tr>
<td>Group 1: 0–5 years of age</td>
<td>Group 2: 6–11 years of age</td>
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<tr>
<td>Hemoglobin levels at day 0, g/dL</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.93</td>
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<tr>
<td>Hemoglobin levels at day 28, g/dL</td>
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<tr>
<td>Mean</td>
<td>10.26</td>
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<tr>
<td>95% CI</td>
<td>(9.95–10.57)</td>
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</table>
* CI = confidence interval.
better results can be obtained using a more favorable sulfonamide in the combination.

A recent publication on molecular markers associated with \textit{P. falciparum} resistance to SP in the Democratic Republic of Congo warned for the irrational of adding As to the SP monotherapy. Our results are in contrast to this suggestion. Further studies on point mutations in the dihydrofolate reductase and dihydropteroate synthase genes should elucidate this controversial point.

Received August 23, 2006. Accepted for publication June 24, 2007.

Acknowledgments: We are grateful to all persons who participated in this study, the staff at the three sites for assistance, and Dr. A. Djimdé (Malaria Research and Training Centre, Bamako, Mali), for technical assistance in the analysis of the PCR samples.

Financial support: This study was supported by a grant from Dafra Pharma NV to Stephen Rulisa.

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