A RANDOMIZED CONTROLLED TRIAL OF ARTEMETHER/BENFLUMETOL, A NEW ANTIMALARIAL AND PYRIMETHAMINE/SULFADOXINE IN THE TREATMENT OF UNCOMPLICATED FALCI PARUM MALARIA IN AFRICAN CHILDREN

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Abstract. We report here the results of a randomized double blind trial comparing artemether and benflumetol, with pyrimethamine/sulfadoxine (P/S). Two hundred eighty-seven children 1–5 years of age with uncomplicated falciparum malaria were enrolled at two centers in The Gambia between July 1996 and December 1996. Following treatment, children were visited at home every 24 hr until a blood film free of asexual parasites was obtained. Genotyping of parasites was used to distinguish recrudescence from new infections. Three days after the start of treatment, 133 (100%) of the CGP56697-treated children compared with 128 (93.4%) of children treated with P/S had cleared their parasites (P = 0.003). The day 15 cure rate was 93.3% for CGP56697 and 97.7% for P/S (P = 0.13). Within the third and fourth week after initiation of therapy, 20 children treated with CGP56697 and one of the P/S-treated children returned with second malaria episodes (P < 0.0001). Genotyping suggested that the majority (19 of 23 [82.6%]) of these second episodes were due to new infections, supporting the World Health Organization recommendation that longer follow-up is not relevant for the assessment of drug efficacy. At the two-week follow-up, 28.9% of the P/S treated children but none of the CGP56697-treated children carried gametocytes (P < 0.0001). This study showed that CGP56697 is safe in African children with acute uncomplicated falciparum malaria, clears parasites more rapidly than P/S, and results in fewer gametocyte carriers. More frequent new infections within the third and fourth week following treatment with CGP56697 than treatment with P/S are likely to be due to the short prophylactic effect of CGP56697.

Malaria is estimated to kill between 1.5 and 2.7 million people every year. An additional 300–500 million people suffer annually from malaria attacks but survive.1 Nine of 10 cases occur in sub-Saharan Africa, where malaria is responsible for more disability adjusted life years than any other disease.2 The main burden of malaria falls on children less than five years of age. The most commonly used treatments, chloroquine and pyrimethamine/sulfadoxine (P/S), have become less effective in the regions where they have been used the longest and are most widely available, such as Southeast Asia. Chloroquine remains useful in selected areas of sub-Saharan Africa, but Malawi, Kenya, and Zambia have been forced to replace chloroquine with P/S as a first-line treatment. However, the efficacy of P/S is already compromised in some parts of Africa. High level resistance of Plasmodium falciparum to P/S has been reported in Tanzania and lower levels of resistance have been recorded in other parts of Africa.3,4 Thus, alternative antimalarial drugs are needed urgently. Atovaquone in combination with proguanil is effective, but like halofantrine and mefloquine, it is too expensive to be used widely in resource poor areas of the world although a donation program is being planned.5 Pyronaridine is highly effective, but has yet to be registered outside China.6 Therefore, great hope is placed on artemisinin derivatives, natural products found in the leafy portions of Artemisia annua (qinghao), a plant used by Chinese herbalists since 168 BC.7 However, the use of short courses of artemisinin derivatives has been limited by their relatively short half-life, which is associated with recurrence of parasitemia following the clearance of P. falciparum. Thus, artemether, the methyl ether of dihydroartemisinin, has been combined with benflumetol, a novel aryl amino alcohol (class II schizontocide) with a relatively slow onset but a half-life of 4–6 days, in an oral formulation known as CGP56697. This drug is undergoing randomized controlled trials in Hainan China, in Bangkok, Thailand, along the Thailand-Myanmar border, in India, and in Ifakara, Tanzania. Following a successful safety trial of CGP56697 in The Gambia,8 we conducted a randomized controlled trial that compared CGP56697 with P/S.

During the safety study of CGP56697, it had been observed that up to one-third of the children returned during the four-week follow-up period with new infections of P. falciparum. A strategy was therefore needed to treat repeated infections, which were unlikely to be resistant to CGP56697. To investigate the efficacy of CGP56697 in repeated malaria episodes, we treated children who returned with falciparum malaria with open label (i.e., the investigator and parent or guardian were aware which drug the child received). CGP56697 and followed their response.

METHODS

The trial design was a randomized, double-blind, controlled efficacy trial that compared CGP56697 with P/S as a treatment for uncomplicated falciparum malaria. The trial was conducted in The Gambia at two centers, one in a semi-urban coastal area and the other in a rural region. Children between one and five years of age were eligible for enrollment if they had a parasitemia of more than 5,000 P. falciparum parasites/μl, a history of fever, lived within 20 km of either trial center, and if a parent or guardian gave informed consent. Children who required parenteral treatment, had been treated within two weeks with P/S, or had a hematocrit

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Eligible children were allocated to receive CGP56697 or P/S by computer-generated block randomization (block size: 4). Investigators and patients remained blinded to the treatment code throughout the study. An emergency code was available to the local safety monitor, but was not used during the trial. Children randomized to CGP56697 received one tablet (20 mg of arteether and 120 mg of benflumetol) at 0, 8, 24, and 48 hr if they weighed less than 15 kg and two tablets at 0, 8, 24, and 48 hr if they weighed 15 or more kg. Children in the P/S group received half a tablet (12.5 mg of pyrimethamine and 250 mg of sulfadoxine) once if they weighed less than 15 kg and one tablet once if they weighed 15 or more kg, in accordance with Gambian government guidelines. Since CGP56697 and P/S differ in size and color, each child received in addition to the active treatment a placebo with the appearance of the drug they were not randomized to receive (double-dummy technique). Children took the starting dose under direct supervision. Children who vomited the starting dose and replacement dose of their antimalarial treatment received chloroquine intramuscularly. Parents or guardians were asked to give their child paracetamol (15 mg/kg) every 6 hr while he or she remained febrile. Children were discharged home and visited every 24 hr by a fieldworker who obtained a blood film at each visit. The fieldworker also recorded the axillary temperature with a digital thermometer, the use of antipyretics during the previous 24 hr, and any adverse experiences. Visits were continued until a blood film free of asexual *P. falciparum* had been collected. Children were examined again two weeks after treatment and a blood film was obtained. After the two weeks follow-up, parents or guardians were encouraged to return with their child if any illness was observed. Symptomatic children with *P. falciparum* parasitemia of 5,000/μl or more were offered re-treatment with CGP56697. Children whose parents or guardians gave consent received the same CGP56697 regimen and were followed as described earlier. The study was approved by the Medical Research Council (MRC) Laboratories Scientific Coordinating and the Gambia Government/MRC Ethical Committees.

The primary trial endpoint was defined as the proportion of children with no detectable *P. falciparum* by day 4. Secondary endpoints were parasite clearance times, day 15 cure rate, and the number of repeat episodes of malaria within 29 days. Children were considered evaluable if no protocol violation was observed and the required blood films could be obtained. Sample size was calculated at 157 children in each study group (significance level = 5%, power = 80%) on the assumption that CGP56697 would give a 100% cure rate by day 4 and P/S a 94% cure rate, as suggested by data obtained previously.

**Laboratory methods.** Thick blood films were stained with Giemsa. The level of parasitemia was calculated on the basis of the number of parasites per 200 leukocytes on a thick film. If gametocytes were present, the count was extended to 1,000 leukocytes and if less than five gametocytes were detected, the count was extended to 2,000 leukocytes. Blood samples for genotyping were obtained from all children at first presentation and, if applicable, at a second presentation. The DNA was purified from the blood collected as described previously. Briefly, parasites were recovered by centrifugation following lysis of the erythrocytes in 0.05% saponin, and the DNA was extracted with phenol/chloroform and recovered by ethanol precipitation. A nested polymerase chain reaction (PCR) protocol was adopted for the analysis of three polymorphic genetic markers from *P. falciparum*: the three sequence families of the merozoite surface protein-1 (MSP-1) block 2 repeat region, the two sequence families of the MSP-2 repeat region, and the II region of glutamate-rich protein (GLURP). The PCR products were stained with ethidium bromide and visualized by UV transillumination following electrophoresis on agarose gels in TBE buffer (100 mM Tris, 100 mM boric acid, 5 mM EDTA). Paired samples were run side by side to allow comparison of the pattern of allelic variants. A recrudescent infection was defined as one that showed a complete match in allelic size for all three genes between the first and second samples. If any clone of a polyclonal primary infection was detected during a second episode, it was considered a recrudescence. The hematocrit of the children was measured by microhematocrit centrifugation.

**Statistical methods.** Normally distributed data were compared using the Student’s *t*-test; homogeneity of variance was tested by the F-test. Discrete data were compared by Fisher’s exact test or the chi-square test with Yates’ correction, as appropriate. Sample size was calculated using the conservative method of Casagrande.

**RESULTS**

A total of 287 children were recruited into the study between July 1996 and January 1997. The 144 children randomized to receive CGP56697 were similar to the 143 children treated with P/S in gender, age, weight, and baseline clinical features (Table 1). Parasites of species other than *P. falciparum* were not detected. The parents or guardians of 57 (19.9%) of the children reported that antimalarial treatment had been given to their child during the previous three months; 34 children (11.8%) had received chloroquine, 16 (5.6%) P/S, four (1.4%) an unknown antimalarial, and three (1.0%) P/S in combination with chloroquine. Prior use of antimalarials was distributed equally between the treatment groups. By day four, 17 children (5.9%) could not be evaluated because they were either lost to follow-up, withdrew their consent, did not comply with the regimen, failed to meet the inclusion criteria, or reported treatment with cotrimoxazole, a potential antimalarial (Figure 1). Two children vomited the starting and replacement dose and received chloroquine injections. One child had received CGP56697 and the other P/S; both children were excluded from the analysis. Another child presented with fever and vomiting 48 hr after having ingested the full dose of P/S. By this time his parasitemia was 40% less than at enrollment and so he received parenteral quinine. The child was thus a treatment failure and was included in the analysis as such. For the reasons indicated in Figure 1 it was not possible to evaluate 25 children (17.4%) treated with CGP56697 and 15 children (10.5%) treated with P/S two weeks after treatment. Children treated with CGP56697 cleared *P. falciparum* significantly faster than children treated with P/S. The time to clear 50% of the parasites was 11.6 hr (95% confidence interval [CI] = 10.8–12.3) for CGP56697-treated children
compared with 21.1 hr (95% CI = 17.3–25.0) for P/S-treated children ($P < 0.0001$). By day 4, all children treated with CGP56697 who could be evaluated had cleared their parasites in contrast with nine P/S-treated children who remained parasitemic (Table 2). Therefore, 133 (100%) of the evaluable children treated with CGP56697 and 128 (93.4%) of the evaluable children treated with P/S were free of parasites three days after treatment (relative risk [RR] = 0.93, 95% CI = 0.89–0.98, $P = 0.003$). If children who were not evaluable are included in the analysis as failures (intention to treat analysis), 133 (92.4%) of 144 CGP56697-treated children compared with 128 (89.5%) of 143 P/S-treated children are considered cured (RR = 0.73, 95% CI = 0.35–1.53, $P = 0.42$). Within 24 hr of treatment with CGP56697, 19 (13.7%) children remained febrile, in contrast with 70 (49.3%) children treated with P/S ($P < 0.001$; Table 3). This more rapid fever clearance was reflected by a lower requirement for antipyretics among CGP56697-treated children compared with P/S-treated children (Table 3). Symptoms such as diarrhea, vomiting, or coughing were observed in a similar proportion of children treated with CGP56697 or P/S.

Two weeks after treatment, eight (6.7%) of 119 CGP56697-treated children who could be evaluated were parasitemic compared with three (2.3%) of the 128 children treated with P/S (Table 2). Therefore, 111 (93.3%) of the 119 CGP56697-treated evaluable children and 125 (97.7%) of the 128 P/S-treated evaluable children were free of parasites (RR = 2.87, 95% CI = 0.78–10.56, $P = 0.13$). In the intention to treat analysis, 111 (77.1%) of 144 CGP56697-treated children compared with 125 (87.4%) of 143 P/S-treated children were considered cured (RR = 1.82, 95% CI = 1.08–3.08, $P = 0.03$). At the time of enrollment, the median hematocrits (Hcts) of children treated with CGP56697 and children treated with P/S were similar (Table 1). Two weeks after treatment, there was still no significant difference in the median Hct between the children who had received CGP56697 (median Hct = 31.9%, interquartile range [IQR] = 29.6–34.0%) and the children who had been treated with P/S (median Hct = 31.8%, IQR = 29.5–35.0%).

Prior to therapy, gametocytes, the sexual stage in the life cycle of P. falciparum, were detected in 4.3% of the children (Table 4). Gametocytes were detected in 2.4% of children three days after treatment with CGP56697 and in none of the children two weeks after treatment. In contrast, the proportion of children with gametocytes increased following treatment with P/S: 13.5% of the evaluated children carried gametocytes three days after P/S treatment and 28.9% carried gametocytes two weeks after treatment.

In addition to the eight children who were found to be parasitemic at the two-week follow-up, another 20 CGP56697-treated children returned to the clinic with signs and symptoms of malaria within four weeks of the initial treatment and were found to be infected with P. falciparum. One child treated with P/S returned during this period. It was possible to compare the genotype of the parasite collected during first and second infections in 23 of the 29 repeat malaria episodes that occurred within 29 days of treatment (Table 5). There was genetic heterogeneity in 19 pairs (83%), suggesting new infections as the source of these second episodes. In four paired samples (17%), the alleles under investigation appeared the same: these infections may have been due to recrudescences. Another 13 children presented between 30 and 64 days after the initial treatment with a further episode of malaria, six had been treated with CGP56697 and seven with P/S. A comparison of isolates from enrollment and second episode showed genetic homogeneity in parasites from one child treated with P/S, which could therefore be a recrudescence; the remaining 12 were heterogeneous and likely to have been new infections.

We detected in 17 of 36 first episodes of malaria infections and in 13 of 36 second episodes of malaria two or more clones of P. falciparum. Overall, 42 children required therapy for second episodes of malaria. The parents or guardians of 34 of these children agreed that their child could be treated with open-label CGP56697. Eight parents whose children had received CGP56697 in the preceding four weeks refused to take part in a further study because of the need for a detailed follow-up (Figure 1). The median time between initial therapy and re-treatment was 25 days (range = 15–64 days). Within four days of re-treatment, six children could not be evaluated because the parents withdrew their consent for the collection of further blood samples and one child was lost to follow-up. All 27 evaluable children, including 19 children treated previously with CGP56697, had cleared their parasites within four days of receiving open-label CGP56697 therapy. Two children whose genotyping was consistent with a recrudescence of P. falciparum cleared the parasites after a second course of CGP56697. Twenty children could be evaluated two weeks after the second treatment; two of the children were found to have a third P. falciparum infection. Four children presented with a third
episode of malaria between 19 and 25 days after re-treatment.

DISCUSSION

Rainfall in The Gambia during 1996 reached only 40% of the yearly average and the number of malaria cases were lower than expected during the malaria season. Despite enrollment below the target sample size, it was possible to detect important differences between children treated with CGP56697 and those treated with P/S. Children with uncomplicated falciparum malaria cleared parasites faster after treatment with CGP56697 than did children treated with P/S. The rapid parasite clearance time observed in this study is within the range seen with five-day courses of other oral artemisinin derivatives (mean = 34-36 hr). Fever is the manifestation of malaria that most frequently causes discomfort. Thus, it was encouraging that children treated with CGP56697 became afebrile significantly faster than children treated with P/S. We measured the requirement for antipy-

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**Figure 1.** Flow chart of the children, indicating their disposition following enrollment into the study. F/U = follow-up. The number of children who could not be evaluated on day 15 includes all children from the start of the trial onwards.

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**Table 2**

Cure rates (absence of parasitemia and symptoms) for children treated with CGP56697 or pyrimethamine/sulfadoxine (P/S) on day 4 and day 15*

<table>
<thead>
<tr>
<th></th>
<th>CGP56697 (95% CI)</th>
<th>P/S (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4 ITT</td>
<td>133/144 = 92.4%</td>
<td>128/143 = 89.5%</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>(86.7-96.1%)</td>
<td>(83.3-94.0%)</td>
<td></td>
</tr>
<tr>
<td>Day 4 evaluable patients</td>
<td>133/133 = 100%</td>
<td>128/137 = 93.4%</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>(97.3-100%)</td>
<td>(87.9-97.0%)</td>
<td></td>
</tr>
<tr>
<td>Day 15 ITT</td>
<td>111/144 = 77.1%</td>
<td>125/143 = 87.4%</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(69.3-83.7%)</td>
<td>(80.8-92.4%)</td>
<td></td>
</tr>
<tr>
<td>Day 15 evaluable patients</td>
<td>111/119 = 93.3%</td>
<td>125/128 = 97.7%</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>(87.2-97.1%)</td>
<td>(93.3-99.5%)</td>
<td></td>
</tr>
</tbody>
</table>

* ITT = intention to treat analysis; CI = confidence interval.
Table 3

<table>
<thead>
<tr>
<th>Day</th>
<th>CGP56697</th>
<th>P/S</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>114/144 (79.2%)</td>
<td>115/143 (80.4%)</td>
<td>0.91</td>
</tr>
<tr>
<td>2</td>
<td>19/139 (13.7%)</td>
<td>70/142 (49.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>6/140 (4.3%)</td>
<td>28/141 (19.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>7/134 (5.2%)</td>
<td>7/141 (5.0%)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Day</th>
<th>CGP56697</th>
<th>P/S</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/140 (4.3%)</td>
<td>6/143 (4.2%)</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>7/134 (5.2%)</td>
<td>8/142 (5.6%)</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>4/119 (3.4%)</td>
<td>12/134 (9.0%)</td>
<td>0.076</td>
</tr>
<tr>
<td>4</td>
<td>1/41 (2.4%)</td>
<td>10/74 (13.5%)</td>
<td>0.094</td>
</tr>
<tr>
<td>15</td>
<td>0/125 (0%)</td>
<td>37/128 (28.9%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Numbers of children who carried gametocytes within two weeks of treatment with CGP56697 or pyrimethamine/sulfadoxine (P/S)
dren in the community. These children can be treated with repeated courses of antimalarials or alternatively the children and their homes might be targeted for prophylactic strategies such as the distribution of impregnated bed nets, eradication of nearby mosquito breeding sites, or the detection and treatment of gametocyte carriers.

We observed a highly significant difference in the development of gametocytomaemia between CGP56697-treated and P/S-treated children. Twenty-nine percent of P/S-treated children carried gametocytes two weeks after treatment, but none of the children treated with CGP56697 carried gametocytes. The effect of CGP56697 and other artemisinin derivatives on gametocytes suggests that this group of drugs may have an effect on malaria transmission. Indeed, some suggestive evidence has been obtained that malaria transmission was reduced in an area of Thailand following the introduction of artemisinin derivatives. The ability of treatment with CGP56697 and artemisinin derivatives to reduce malaria transmission will be determined by the contribution of the treated patients to the overall reservoir of infection. The transmission blocking potential of CGP56697 in different settings and the way in which this drug achieves its effect on gametocytes require further studies.

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