Artesunate Reduces but Does Not Prevent Posttreatment Transmission of \textit{Plasmodium falciparum} to \textit{Anopheles gambiae}

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Combination therapy that includes artemisinin derivatives cures most falciparum malaria infections. Lowering transmission by reducing gametocyte infectivity would be an additional benefit. To examine the effect of such therapy on transmission, Gambian children with \textit{Plasmodium falciparum} malaria were treated with standard regimens of chloroquine or pyrimethamine-sulfadoxine alone or in combination with 1 or 3 doses of artesunate. The infectivity to mosquitoes of gametocytes in peripheral blood was determined 4 or 7 days after treatment. Infection of mosquitoes was observed in all treatment groups and was positively associated with gametocyte density. The probability of transmission was lowest in those who received pyrimethamine-sulfadoxine and 3 doses of artesunate, and it was 8-fold higher in the group that received pyrimethamine-sulfadoxine alone. Artesunate reduced posttreatment infectivity dramatically but did not abolish it completely. The study raises questions about any policy to use pyrimethamine-sulfadoxine alone as the first-line treatment for malaria.

Treatment of malaria in much of sub-Saharan Africa still relies heavily on chloroquine, which is the only available and affordable drug for many countries. Chloroquine is, however, no longer effective in many areas of Africa because of the high level of resistance that has been acquired by \textit{Plasmodium falciparum}. Chloroquine resistance has been linked to an increase in malaria-related childhood mortality [1].

Pyrimethamine-sulfadoxine has already replaced chloroquine as first-line treatment for malaria in several African countries, but pyrimethamine-sulfadoxine resistance is developing rapidly [2], probably as a consequence of its long half-life [3]. In response to this problem of growing drug resistance in Africa, combination therapy using drugs with different modes of action and different pharmacologic properties has been proposed [4]. The basic premise behind the use of combination therapy is that the chance of a mutant parasite emerging that is simultaneously resistant to 2 antimalarial drugs is very low. The use of an artemisinin derivative that has a very short half-life, such as artesunate, in combination with longer-acting agents, such as pyrimethamine-sulfadoxine, means that 1 drug protects the other against the risk of a resistant strain emerging [4].

The rate of spread of resistance could be reduced further if the drug(s) also had an effect on gametocytes, the sexual stage of the malaria parasite responsible for infection of the mosquito. Studies in Thailand [5] showed that when artemisinin derivatives were introduced as a component of first-line treatment, there was a significant reduction in the incidence of clinical \textit{P. falciparum} malaria during the next 2 years. Furthermore, studies in The Gambia [6] and Tanzania [7] gave a strong indication that artemisinin derivatives—in this case, co-artemether (artemether combined with lumefantrine)—reduce the transmissibility of \textit{P. falciparum}. More recent studies in The Gambia, which assessed the use of artesunate in combination with pyrimethamine-sulfadoxine, showed markedly lower gametocyte prevalence after treatment with the combination than after treatment with pyrimethamine-sulfadoxine alone [8, 9]. It remains unclear from these studies whether the observed reduction in gametocyte numbers is due to the gametocidal effect of artemisinins, to the lower production of gametocytes due to rapid reduction in the total parasite burden, or to a combination of both. More important, it is not known whether the remaining gametocytes are subsequently infective to mosquitoes.

Although clinical cure rates of antimalarial drugs can be measured in human subjects, the gametocidal effect of a treatment has to be evaluated in mosquito-feeding experiments. Therefore, we compared the effects of treatment of uncomplicated malaria with chloroquine, with pyrimethamine-sulfadoxine alone, and with combinations of artesunate and pyrimethamine-sulfadoxine.
on the posttreatment infectivity of gametocytes to mosquitoes and, hence, on the transmissibility of the disease.

**Patients and Methods**

*Study children and treatment.* The study was conducted over 2 years. Children attending the health center at Farafenni, The Gambia (a rural town 170 km from the coast), were recruited, using a common protocol, between September and December 1998 and during a similar period in 1999. Eligible children had a body weight >5 kg, a history of fever, and parasitemia >500/μL of blood. Exclusion criteria included severe anemia (hemoglobin <5 g/dL, packed cell volume <15%); any other signs or symptoms of severe malaria; inability to take drugs orally; treatment with pyrimethamine-sulfadoxine within the past 2 weeks; and any evidence of chronic disease or other acute infection.

Children were randomized to receive chloroquine or pyrimethamine-sulfadoxine alone or with artesunate. Children in the chloroquine group were orally administered 10 mg of chloroquine base per kilogram body weight for 3 days. Children in the 3 other groups received one-half tablet of pyrimethamine-sulfadoxine (12.5 mg pyrimethamine–250 mg sulfadoxine) for a body weight of ≤10 kg and a further one-quarter tablet for each 5 kg increase in weight. Children in the pyrimethamine-sulfadoxine-artesunate groups received pyrimethamine-sulfadoxine plus either a 4 mg/kg immediate dose of artesunate or 4 mg/kg given once daily for 3 days. All children were observed for 1 h after the first dose, and any child who vomited was administered a replacement dose. Starting and replacement doses were administered under supervision. Doses of chloroquine or artesunate on days 1 and 2 after presentation were directly observed at the child’s home by a trained field worker. Each child received 10 mg/kg paracetamol under supervision, and parents or guardians were instructed to administer further doses every 6 h until the child’s symptoms had subsided. Open-label chloroquine (Alkaloida), pyrimethamine-sulfadoxine (Pharma-med), and paracetamol (Echo International Services) were used. Artesunate was manufactured by Guilin Pharmaceutical Works and was supplied by Sanofi, France.

Trial clinicians were aware of which drug each child had received but could not influence the choice of drug and were not involved in subsequent laboratory stages of the study. Children were asked to return to the Medical Research Council (MRC) Field Station at Farafenni 4 (in 1998) or 7 (in 1999) days after treatment. Field workers made further visits to the children on posttreatment days 14 and 28. Treatment with the pyrimethamine-sulfadoxine-artesunate combinations in the 1998 season was possible only from the beginning of November because of the requirement to first complete a safety trial [8]; thus, children recruited in September and October 1998 were treated with chloroquine or with pyrimethamine-sulfadoxine alone. In 1999, 2 treatment regimens, pyrimethamine-sulfadoxine plus artesunate for 3 days and pyrimethamine-sulfadoxine alone, were randomly assigned at a ratio of 4:1, respectively. This ratio was based on the gametocyte prevalence on posttreatment day 7 for the previous year (figure 1A). The rationale was that the recruitment of 500 individuals would provide 80 (divided equally between the 2 groups) who were gametocytemic on day 7.

*Laboratory samples.* Thick-blood film, hematocrit, and microtainer samples were obtained from patients on recruitment (day 0). The samples were read on slides stained with Field’s stain, and blood films then were returned to the laboratory and were allowed to dry for 24 h. The samples then were stained with Giemsa stain, and 100 fields were screened for asexual and sexual stage parasites. Parasitemia was calculated both on the basis of parasites per 200 leukocytes and, for gametocytes, on the basis of parasites per 1000 leukocytes and was expressed as parasites per microliter [10].

On day 4 (1998) or day 7 (1999) after treatment, patients returned to the MRC in Farafenni, where samples for thick-blood film and hematocrit were obtained. Thick-blood films were stained with Field’s stain, and 100 fields were examined for the presence of parasites. These slides were reread later after staining with Giemsa.

On day 14, thick-blood film and microtainer samples were obtained...
and were processed as on day 0. Thick-blood film samples also were obtained on posttreatment day 28 in the 1998 study. For paired data for the 14-day follow-up during the 1999 season.

**Membrane feeding and mosquito dissection.** This technique involves feeding mosquitoes on blood samples via an artificial membrane attached to a water-jacketed glass feeder maintained at 37°C. Blood samples were obtained from children who were gametocytemic (limit of detection, 5 gametocytes/µL) on day 4 (1998) or 7 (1999) and had hemoglobin counts >8 g/dL. The feeds were performed as described elsewhere [11]. In brief, venous blood in citrate–phosphate dextrose was centrifuged, and the plasma was removed. After being washed, the red blood cell pellet was resuspended to a hematocrit of 33% in the original (autologous) plasma or in a pool of AB control serum samples from nonexposed European donors. Each suspension then was fed to 3–5-day-old female *Anopheles* mosquitoes. The mosquitoes that were used were reared throughout the study as the first-generation progeny of local mosquitoes caught in areas where *Anopheles gambiae sensu stricto* predominates. Mosquito midguts were dissected out 7–8 days later, and the number of oocysts—a developmental stage of the parasite found on the insect midgut—was recorded.

**Analyses.** Three measures were used to determine outcome: (1) infectious proportion (i.e., the proportion of children’s blood samples that infected any mosquitoes), (2) the prevalence of mosquito infection (i.e., the proportion of mosquitoes that developed oocysts), and (3) the intensity of mosquito infection (i.e., the arithmetic mean oocyst count in all fed mosquitoes).

The prevalence of mosquito infection was compared among groups, using logistic regression, after weighting the observations to account for the correlation among counts within subjects [14]. Stata statistical software (version 6; Stata) was used for the analyses [15].

**Results**

In 1998, 472 children who fulfilled the study inclusion criteria were enrolled and were randomly assigned among 3 treatment groups, and in 1999, 500 children were assigned to 2 treatment groups (table 1). In 1998, children treated with pyrimethamine-sulfadoxine-artesunate were recruited later in the malaria season (November to December) and had lower mean parasite densities than did those treated with chloroquine or pyrimethamine-sulfadoxine alone who were recruited throughout the season, but particularly in the early months (September to October; data not shown). The average age (table 1) and weight (17.7 kg) of participants were similar, and the rate of recruitment was uniform throughout the study period. There was a clear indication that children treated later in the season were more likely to have circulating gametocytes at the time of treatment than those recruited earlier (table 1). Since the primary objective of the study was to determine whether any of the drugs would affect developing gametocytes and hence their infectivity, children who were gametocytemic on the day of treatment were excluded, because mature gametocytes are known to be unaffected by the drugs used.

Figure 1A shows the gametocyte prevalence rates in the 1998 study over the 28-day follow-up period. Figure 2A shows comparable data for the 14-day follow-up during the 1999 season. Gametocyte densities in gametocytemic individuals for 1998 and 1999 are shown in figures 1B and 2B, respectively.

Transmission of *P. falciparum* to mosquitoes was studied in 158 subjects over the 2 years. Table 1 shows the gametocyte

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**Table 1.** Characteristics of children enrolled in studies of the posttreatment malaria infectivity effects of chloroquine, pyrimethamine-sulfadoxine (PSD) alone, and PSD plus 1 (PSD + A1) or 3 (PSD + A3) doses of artemesunate.

<table>
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<tr>
<td>Subjects at enrollment</td>
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<tr>
<td>No. with gametocytes/total no. (%)</td>
<td>3/120 (2.5)</td>
<td>152/249 (6.0)</td>
<td>111/103 (11)</td>
<td>6/100 (6.0)</td>
<td>32/400 (8.0)</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
<td>6.4 (0.9–19)</td>
<td>6.4 (0.9–18)</td>
<td>6.5 (0.9–16)</td>
<td>4.6 (0.6–10)</td>
<td>5.0 (0.8–10)</td>
</tr>
<tr>
<td>Mean PCV, % (range)</td>
<td>31 (15–41)</td>
<td>31 (15–43)</td>
<td>29 (15–42)</td>
<td>31 (19–46)</td>
<td>31 (14–44)</td>
</tr>
<tr>
<td>Subjects chosen for experiments a</td>
<td>18</td>
<td>49</td>
<td>15</td>
<td>44</td>
<td>32</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
<td>6.7 (2.0–16)</td>
<td>6.6 (1.2–18)</td>
<td>7.4 (1.9–14)</td>
<td>4.2 (1–10)</td>
<td>4.1 (1.9–9)</td>
</tr>
<tr>
<td>Mean PCV, % (range)</td>
<td>30 (15–38)</td>
<td>30 (16–39)</td>
<td>27 (17–33)</td>
<td>31 (21–46)</td>
<td>28 (15–39)</td>
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<tr>
<td>Median gametocyte density/µL on day of sampling (IQR)</td>
<td>25 (10–196)</td>
<td>76 (32–350)</td>
<td>16 (10–48)</td>
<td>100 (10–334)</td>
<td>5 (5–10)</td>
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<td>Transmission experiments</td>
<td></td>
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<tr>
<td>Children infectious, %</td>
<td>61</td>
<td>41</td>
<td>53</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td>Mean prevalence of mosquito infection, % (range)</td>
<td>9.8 (0–65)</td>
<td>4.5 (0–76)</td>
<td>9.7 (0–56)</td>
<td>4.3 (0–45)</td>
<td>2.5 (0–14)</td>
</tr>
<tr>
<td>Intensity (range) of mosquito infection b</td>
<td>1.4 (0–25)</td>
<td>0.45 (0–16)</td>
<td>0.65 (0–7.5)</td>
<td>0.61 (0–13.5)</td>
<td>0.07 (0–1.1)</td>
</tr>
</tbody>
</table>

**NOTE.** IQR, interquartile range; PCV, packed cell volume.

* a That is, chosen for experiments on malaria transmission.

* b Samples were for mosquito feeding. Feeding was performed 4 days after treatment in 1998 and 7 days after treatment in 1999.

* c Intensity of mosquito infection is the arithmetic mean oocyst count (range of within-subject means).
densities in the patient blood samples used for membrane feeding. There was significant variation among treatment groups in the distribution of gametocyte densities (Kruskal-Wallis test \( \chi^2, 37; 9; 3 \text{ df; } P < .001 \)). The median density was lowest in the group that received pyrimethamine-sulfadoxine plus 3 doses of artesunate and was highest in the group that received pyrimethamine-sulfadoxine alone. The distribution of gametocyte density in those treated with pyrimethamine-sulfadoxine alone was similar between 1998 and 1999 (Kruskal-Wallis test \( \chi^2, 1.4; 1 \text{ df; } P = .23 \)).

Table 1 shows the proportion of children from each group who were infectious. A total of 7014 mosquitoes were dissected; the mean number per subject was 42 (range, 6–83). In each group, there was no significant difference overall between auxological plasma and control serum samples (data not shown); thus, data for each individual were pooled. There was a positive association between prevalence of infection in mosquitoes and gametocyte density in each treatment group (data not shown). When compared with transmission after treatment with pyrimethamine-sulfadoxine, with gametocyte density controlled for, the prevalences of mosquito infection were significantly higher in mosquitoes fed on blood samples from children treated with chloroquine \( (P = .002; \text{ odds ratio [OR], } 3.7; 95\% \text{ confidence interval [CI], } 1.6–8.2) \) or with pyrimethamine-sulfadoxine plus 1 dose of artesunate \( (P = .001; \text{ OR, } 4.6; 95\% \text{ CI, } 1.9–11) \).

The mean intensity of oocyst infection was significantly lower in the group treated with pyrimethamine-sulfadoxine plus 3 doses of artesunate \( (P = .002; \text{ table 1}) \). However, this reflected the low gametocyte density (mostly \(<10/\mu\text{L} \)) in children administered 3 doses of artesunate, and the difference according to treatment was not seen after adjusting for the effect of gametocyte density (data not shown). Gametocyte densities \( \geq 99/\mu\text{L} \) produced higher density oocyst infections in the other 3 groups.

**Discussion**

Treatment of children with acute *P. falciparum* malaria with pyrimethamine-sulfadoxine plus artesunate reduced the prevalence and density of gametocytes in the days after treatment. One objective of the study was to determine whether artesunate is gametocyticidal during the long period (8–10 days) of gametocyte development and maturation \([16, 17]\) and might therefore reduce or prevent transmission. Some of the children were found to have gametocytes on presentation, and, as expected, artesunate had no effect on these, since most of the children were still gametocytemic and infectious 7 days later (data not shown). It has been reported that artemisinin derivatives are gametocyticidal \([18, 19]\), and the significantly lower gametocyte prevalence and density following treatment with artesunate combinations supports the conclusion that there is some effect of artesunate on sexual stages of the parasite. Even after children who had detectable gametocytes on presentation were excluded, it was clear that some immature stages of gametocytes were unaffected by artesunate. They continued to develop to maturity, and their infectivity was \(~40\% \), irrespective of whether a 1- or 3-dose artesunate regimen was used. Thus, artesunate treatment affects transmission (1) by adversely affecting the very young gametocyte forms and (2) as a consequence of the speed and effectiveness with which the asexual forms are killed \([5]\). Chloroquine impairs development of only the very youngest gametocytes \([20]\). Contrary to findings in other infectivity studies \([21, 22]\), we found no evidence of enhanced gametocyte infectivity following chloroquine treatment; however, our preliminary observations, which are in line with observations in Senegal \([23]\), suggest that chloroquine-resistant parasites may be more infectious (data not shown).

The effect of treatment with pyrimethamine-sulfadoxine on
the prevalence of gametocytes was dramatic. We found a 3-fold difference in gametocyte prevalence and a 5–6-fold difference in gametocyte density after treatment with pyrimethamine-sulfadoxine alone, compared with chloroquine or with either of the pyrimethamine-sulfadoxine-artesunate combinations. This finding is in agreement with those of recent efficacy studies in The Gambia [8, 9] and Senegal [23]. There are a number of possible explanations for the large increase in gametocyte numbers after pyrimethamine-sulfadoxine treatment. Since this increase was seen as early as 4 days after treatment, the gametocytes already were developing when the drug was administered. Thus, this was not a pyrimethamine-sulfadoxine–induced production of gametocytes—the time scale was too short—but perhaps was a drug-induced release or redistribution of gametocytes that is not seen with the other treatments. It is intriguing and important that, although pyrimethamine-sulfadoxine alone induces massive release of gametocytes, the pyrimethamine-sulfadoxine-artesunate combination does not. The relatively lower infectivity per gametocyte may be due to a pyrimethamine-sulfadoxine–triggered release of immature forms that are sequestered away from the peripheral blood and are not yet fully infectious. An alternative hypothesis is that pyrimethamine-sulfadoxine has no impact on gametocyte dynamics, and the observed prevalence and density are those that would occur normally after an untreated malaria attack. We observed no differences in transmission parameters between blood feeds using either autologous or control serum samples after pyrimethamine-sulfadoxine treatment, which indicates that there was no residual effect of pyrimethamine on parasite development in the mosquito, as others have proposed [21].

During the time when gametocytes are circulating in the blood (≤20 days), they will have a period when they are most infectious, which is preceded and followed by periods of lower infectivity. Sexual stage–specific immunity may also have an effect on transmission. Together, these 2 factors may explain why high densities of gametocytes sometimes failed to produce infections in mosquitoes, although the likelihood of transmission generally increased with higher gametocyte density [24]. For our assessment of infectivity, we used children with patent gametocytomias. Other studies document mosquito infection in individuals with undetectable gametocytes [25]. The relationship between gametocyte density and infectivity suggests that an effect of subpatency would not be large but could influence the outcome, especially with the pyrimethamine-sulfadoxine–3-dose artesunate group. We are currently investigating the prevalence of subpatent gametocytemia, using reverse transcriptase–polymerase chain reaction.

Since the infectivity of gametocytes assessed 4 days after treatment in 1998 and 7 days after treatment in 1999 was essentially the same, we used these data, together with the prevalence of gametocytemia on day 7, to estimate the proportion of infectious individuals and the probability of transmission after treatment with each drug regimen (table 2). Gametocyte density did not come into this calculation, since we were interested only in whether a treated person can transmit the infection. These estimates were lowest in the group that received 3 doses of artesunate and were, respectively, 5- and 9-fold higher in the group that received pyrimethamine-sulfadoxine alone (table 2). In essence, the higher prevalence of gametocytemia in the pyrimethamine-sulfadoxine group more than offsets the lower risk of transmission from individual children treated with this drug.

There is obvious benefit to be derived, in relation to the subsequent transmissibility of the disease, from using the pyrimethamine-sulfadoxine-artesunate combination rather than pyrimethamine-sulfadoxine alone for treatment of malaria. In an area endemic for malaria, such as The Gambia, symptomatic children represent only a small part of the reservoir from which mosquitoes acquire infection [26]. When there has to be a change in the recommended first-line treatment, with consequent much wider use of the drug, treatment efficacy and impact on transmission should both be considered. Despite the initial higher cost of the drug combination, it is reasonable to expect that lower gametocyte prevalence and infectivity should result in less transmission, fewer reinfections, and a decrease in the number of treated malaria episodes. On the basis of these results, use of the pyrimethamine-sulfadoxine-artesunate combination, if not ideal, is certainly preferable to the use of pyrimethamine-sulfadoxine alone.

### Table 2. Estimates of posttreatment infectivity of children treated with different antimalarial regimens.

<table>
<thead>
<tr>
<th>Antimalarial regimen</th>
<th>No. (%) of children with gametocytemia on day 7</th>
<th>No. (%) infectious</th>
<th>Prevalence of mosquito infection, %</th>
<th>Children infectious after treatment, %</th>
<th>Average probability of transmission, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSD + A3</td>
<td>328 (13)</td>
<td>32 (38)</td>
<td>2.5</td>
<td>4.9</td>
<td>0.3</td>
</tr>
<tr>
<td>PSD + A1</td>
<td>70 (13)</td>
<td>15 (53)</td>
<td>9.7</td>
<td>6.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>89 (20)</td>
<td>18 (61)</td>
<td>9.8</td>
<td>12.2</td>
<td>2.0</td>
</tr>
<tr>
<td>PSD</td>
<td>255 (61)</td>
<td>83 (42)</td>
<td>4.4</td>
<td>25.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

**NOTE.** A1, 1 dose of artesunate; A3, 3 doses of artesunate; PSD, pyrimethamine-sulfadoxine.

a Excludes those who had gametocytes on day 0.

b Percentage of infectious gametocytes multiplied by percentage of children with gametocytes; that is, (second column × third column)/100 = fifth column.

c Percentage of infected mosquitoes multiplied by percentage of children with gametocytes; that is, (second column × fourth column)/100 = sixth column.
Acknowledgments

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References