Activities of Artesunate and Primaquine against Asexual- and Sexual-Stage Parasites in Falciparum Malaria

Sasithon Pukrittayakamee,1 Kesinee Chotivanich,1 Arun Chantra,1 Ralf Clemens,1 Sornchai Looareesuwan,1 and Nicholas J. White1,2,*

Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,1 and Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom2

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The activities of primaquine in combination with quinine or artemisunate against asexual- and sexual-stage parasites were assessed in 176 adult Thai patients with uncomplicated Plasmodium falciparum malaria. Patients were randomized to one of the six following 7-day oral treatment regimens: (i) quinine alone, (ii) quinine with tetracycline, (iii) quinine with primaquine at 15 mg/day, (iv) quinine with primaquine at 30 mg/day, (v) artemisunate alone, or (vi) artemisunate with primaquine. Clinical recovery occurred in all patients. There were no significant differences in fever clearance times, rates of P. falciparum reappearance, or recurrent vivax malaria between the six treatment groups. Patients treated with artemisunate alone or in combination with primaquine had significantly shorter parasite clearance times (mean ± standard deviation = 65 ± 18 versus 79 ± 21 h) and lower gametocyte carriage rates (40% versus 62.7%) than those treated with quinine (P < 0.007). Primaquine did not affect the therapeutic response (P > 0.2). Gametocytosemia was detected in 98 patients (56% [22% before treatment and 34% after treatment]). Artesunate reduced the appearance of gametocytosemia (relative risk [95% confidence interval] = 0.34 [0.17 to 0.70]), whereas combinations containing primaquine resulted in shorter gametocyte clearance times (medians of 66 versus 271 h for quinine groups and 73 versus 137 h for artemisunate groups; P < 0.038). These results suggest that artemisunate predominantly inhibits gametocyte development whereas primaquine accelerates gametocyte clearance in P. falciparum malaria.

Multidrug-resistant Plasmodium falciparum malaria is of increasing public health concern in tropical countries. Combination regimens of two antimalarial drugs with different targets of action have been shown to delay the development of drug resistance and to improve cure rates in falciparum malaria (12, 23). Combination treatments with quinine-tetracycline, artemisunate-tetracycline, or artemether-lumefantrine are efficacious worldwide, providing cure rates of 90 to 100% (9, 17, 23). In children and pregnant women, for whom tetracyclines are contraindicated, quinine-clindamycin is an effective alternative to quinine-tetracycline (10, 16), although adherence to the 7-day quinine regimens is often poor. Combination treatments which include an artemisinin derivative are efficacious in 3-day regimens and have the additional benefit of reducing gametocyte carriage and thus reducing transmission potential (14, 13, 21, 22).

Primaquine, the only generally available 8-aminoquinoline antimalarial drug, has been used for half a century as a hypnozoitocidal drug against Plasmodium vivax malaria, as a causal prophylactic against all malaria species, and as a gametocytocidal drug against P. falciparum malaria (3, 6). The World Health Organization has recommended for some areas that primaquine, in a single dose, be added to treatment regimens for falciparum malaria to reduce the transmissibility of the infection (25). Primaquine at hypnozoitocidal doses is also effective against asexual stages of P. vivax malaria (15, 18). The aim of the present study was to assess the value of adding primaquine to artemisunate or quinine compared with the standard 7-day oral quinine-tetracycline regimen.

MATERIALS AND METHODS

Patients. This prospective study was conducted with adult male patients with acute uncomplicated P. falciparum malaria admitted to the Bangkok Hospital for Tropical Diseases, Bangkok, Thailand. Fully informed consent was obtained from each subject. Exclusion criteria were patients with severe malaria (24) or patients with primary mixed malaria infections. Patients who gave a history of drug hypersensitivity, who had taken any antimalarial drugs within the previous 48 h, or whose urine was positive in screening tests for sulfonamides (lignin test) or 4-aminoquinolines (Wilson-Edeson test) were also excluded. Patients with G6PD deficiency were excluded from treatment with primaquine. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Management. After clinical assessment and confirmation of the diagnosis from thick and thin blood smears, baseline blood samples were taken for routine hematology and biochemistry. Patients were randomized to 7-day treatment with one of the six following oral regimens: (i) quinine sulfate (Thai Government Pharmaceutical Organization; 300 mg of salt/tablet) at 10 mg of salt/kg of body weight three times a day for 7 days, (ii) quinine sulfate (10 mg of salt/kg three times a day) in combination with tetracycline (Thai Government Pharmaceutical Organization; 250 mg/tablet) at 4 mg/kg four times a day for 7 days, (iii) primaquine sulfate (10 mg of salt/kg three times a day) in combination with primaquine (Thai Government Pharmaceutical Organization; 15 mg of base/tablet) at 0.25 mg of base/kg daily (adult dose, 15 mg of base/day) for 7 days, (iv) quinine sulfate (10 mg of salt/kg three times a day) in combination with primaquine at 0.50 mg of base/kg daily for 7 days, (v) artemisunate (Guilin No. 1 Factory, Guangxi, People’s Republic of China; 50 mg of salt/tablet) at 3.3 mg/kg (adult dose, 200 mg) on the first day and then 1.65 mg/kg (adult dose, 100 mg/day) for a further 6 days, and (vi) artemisinin (3.3 mg/kg on the first day and then 1.65 mg/kg for a further 6 days) in combination with primaquine at 0.50 mg of base/kg daily for 7 days. Oral acetylsalicylic (0.5 to 1 g every 8 h) was given for fever of >38°C. Vital signs were recorded every 4 h until resolution of fever and thereafter every 6 to 12 h. The fever clearance time (FCT) was defined as the time taken for the body
RESULTS

Patients. The study included 176 male patients with *P. falciparum* malaria, aged between 14 and 62 (mean ± standard deviation [SD] = 24 ± 9) years. Patients were randomized to one of six oral treatment regimens with quinine or artesunate or combined therapy with primaquine or quinine-tetracycline as detailed in Table 1. The majority of patients (n = 143; 81%) came from the western border of Thailand, where the predominant multidrug-resistant *P. falciparum* is prevalent. More than half of the patients had a history of previous malaria infection (n = 91; 52%). There were no significant differences in admission parasite counts between the quinine groups (P = 0.33), but patients who received artesunate alone had significantly higher baseline parasitemias than those treated with artesunate-primaquine (P = 0.021) (Table 1). Between the six treatment groups, there were no significant differences in rates of previous malaria infection or other baseline laboratory data. Elevated serum bilirubin (total bilirubin of ≳3 mg/dl) was noted in 30 patients from all groups. None of the studied patients had other complications, and all patients with hyperbilirubinemia had normal bilirubin levels by day 14.

Clinical responses. Clinical recovery following treatment occurred in all patients, and none developed severe malaria (Table 1). The overall median (range) FCT was 46 (7 to 180) h and was not significantly different between patients treated with the artesunate regimens (33 [7 to 180] h) and those treated with the quinine regimens (50 [7 to 154] h) (P = 0.10). Between the four quinine groups, the combined therapies yielded shorter FCTs than quinine alone, but this was statistically significant
only for the quinine-tetracycline group ($P < 0.001$). Patients treated with artesunate either alone or in combination with primaquine had similar FCTs ($P = 0.92$). None of the studied patients developed allergic rashes or other serious adverse effects as monitored by clinical symptoms and laboratory data (data not shown).

Parasitological responses. The overall mean PCT ± SD was 75.4 ± 21.5 h and was significantly shorter in the artesunate groups (65 ± 18 h) than in the quinine groups (79 ± 21 h) ($P < 0.001$) (Fig. 1). There was no significant difference in PCT between the quinine subgroups ($P = 0.93$) or between the artesunate subgroups ($P = 0.26$). The parasite reduction ratios at 48 h (parasite count on admission/parasite count at 48 h) were significantly higher in the artesunate groups (median range = 798 [59 to 12,521]) than in the quinine groups (136 [0.2 to 8,895]) ($P < 0.001$). There was no significant difference in the 48-h parasite reduction ratio between the quinine subgroups ($P = 0.19$) or between the artesunate subgroups ($P = 0.55$).

Clinical course. Overall, 142 (84%) of the recruited patients completed at least 28 days of follow-up or remained in the hospital until appearance of vivax or falciparum malaria (Table 2). Of these 142 patients, 23 (16%) had subsequent reappearance of $P$. falciparum malaria and another 22 (16%) had delayed appearance of vivax malaria. Among the six treatment groups, there were no significant differences in recrudescence rates ($P = 0.16$) or rates of recurrent $P$. vivax infection ($P = 0.33$). The overall cure rate (no subsequent appearance of $P$. falciparum malaria) was 84%, and it ranged from 100% in the quinine-tetracycline group to 72% in the quinine-primaquine group. The cure rate in the quinine-tetracycline group was

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Completed follow-up</th>
<th>Subsequent appearance of:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>$P$. falciparum</td>
</tr>
<tr>
<td>Quinine</td>
<td>25</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Quinine + tetracycline</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Quinine + primaquine (0.25 mg/day)</td>
<td>18</td>
<td>5 (28)</td>
</tr>
<tr>
<td>Quinine + primaquine (0.50 mg/day)</td>
<td>31</td>
<td>8 (7)</td>
</tr>
<tr>
<td>Artesunate</td>
<td>21</td>
<td>2 (9.5)</td>
</tr>
<tr>
<td>Artesunate + primaquine</td>
<td>25</td>
<td>4 (16.0)</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>23 (16.2)</td>
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</tbody>
</table>
Gametocyte detection rates after the different treatments were not significantly different between the two artesunate groups; the addition of primaquine to the two other drugs resulted in a significant reduction in gametocyte carriage (odds ratio [95% confidence interval] = 0.83 [0.20 to 0.42]; P = 0.009).

**Gametocytemia.** Circulating gametocytes were detected in 98 patients (56%) from all groups (in 39 patients before treatment and in 59 after initiation of treatment) (Table 3). The overall gametocyte detection rate on admission was 22% (n = 39), and it was not significantly different between the six treatment groups (P = 0.88). Following treatment, the emergence of gametocytes was significantly less frequent in the artesunate group than the quinine groups (14 versus 47%) (relative risk [95% confidence interval] = 0.34 [0.17 to 0.70]; P < 0.001). The gametocyte detection rates after the different treatments were not significantly different between the two artesunate groups (P = 0.80). Among quinine-treated patients, the overall rate of gametocytemia was lower in the high-dose quinine-primaquine group than in the normal-dose quinine-primaquine group or the quinine-tetracycline group (P = 0.045). By stratified analysis, the addition of primaquine to the other two drugs resulted in a significant reduction in gametocyte carriage rates (odds ratio [95% confidence interval] = 0.42 [0.20 to 0.83]; P = 0.009).

**Duration of gametocyte carriage.** Transmission potential is considered to have insignificant activity against asexual stages of *P. falciparum* (20), although there have been uncertainties as to whether or not this results from resistance. Primaquine does have significant activity against sexual blood stages in *P. vivax* malaria, but the activity is weaker than those of other major antimalarial drugs (15). A new long-acting 8-aminoquinoline, tafenoquine (19), has been shown in vitro to be more effective than primaquine-primaquine groups as one) in a two-way analysis of variance, primaquine was associated with a significant shortening of GCTs (P < 0.001) but artesunate was not (P = 0.25).

There were no significant correlations between GCT and PCT either within the quinine or artesunate groups or within the three combined primaquine regimens (r = 0.20; P = 0.27).

**DISCUSSION**

The primary objective of antimalarial treatment is to cure the infection, but an important secondary objective is to prevent transmission. Primaquine has unique multiple-stage activity against malaria parasites. In 1951, primaquine was selected as the most active and least toxic hypnozoitocidal drug among the 8-aminoquinoline series (5). The causal prophylactic and gametocytocidal effects of primaquine in *P. falciparum* were characterized later (6). Since then primaquine has been recommended and used as a transmission-blocking agent in falciparum malaria, albeit with little evidence that this policy has a significant effect on the incidence of malaria at the community level. Primaquine is considered to have insignificant activity against asexual stages of *P. falciparum* (20), although there have been uncertainties as to whether or not this results from resistance. Primaquine does have significant activity against sexual blood stages in *P. vivax* malaria, but the activity is weaker than those of other major antimalarial drugs (15). A new long-acting 8-aminoquinoline, tafenoquine (19), has been shown in vitro to be more effective than primaquine-

![FIG. 2. Mean gametocyte carriage (in person-hours) in patients with *P. falciparum* malaria treated with artesunate or quinine in the presence and absence of primaquine.](http://aac.asm.org/)
quine against asexual stages of malaria parasites and is still under investigation for the treatment and prophylaxis of falciparum malaria infection.

In the present study, primaquine in combination with quinine or artesunate had no additional significant effects on the activity of either drug against asexual blood stages as assessed by PCT and PCT. This confirms earlier in vivo studies indicating a lack of activity against blood stages in falciparum malaria (1). Only the quinine-tetracycline regimen gave a 100% cure rate, which is significantly better than those of the other quinine-containing regimens. In areas where malaria is endemic, mixed infection with *P. falciparum* and *P. vivax* is common, and mixed infection is found in over 30% of patients coming from the border areas of Thailand where malaria transmission is high (8, 15, 18). However, 7-day regimens of primaquine as used here are probably not sufficient to eradicate the hypnozoites of *P. vivax* (2).

Artemisinin derivatives are the most active of the antimalarial drugs against the asexual blood stages of malaria parasites. They also reduce gametocyte carriage in *P. falciparum* infections (4, 13, 14, 22). In recent field studies in Thailand, this effect has been greater than that with primaquine (21). In the present study, artesunate with or without primaquine was more rapidly acting than the quinine regimens as assessed by PCTs, although recrudescence rates and rates of cryptic vivax infections in the artesunate groups were not significantly different from those in the quinine groups. Patients treated with artesunate alone had significantly lower gametocyte carrier rates (44%) than those treated with the other nonprimaquine regimens (57% for quinine and 77% for quinine-tetracycline). Artesunate effectively prevented the appearance of gametocytemia. The average GCT with artesunate alone (median = 138 h) was also shorter than that with quinine or quinine-tetracycline (216 and 288 h, respectively), but there was considerable variation and these differences were not statistically significant. The mechanism whereby artemisinins reduce *P. falciparum* gametocytemia in vivo (4, 13, 14, 21, 22) and in vitro (7, 11) has not been fully elucidated. The artemisinin derivatives could act in three linked ways: they may possess gametocytocidal effects against more mature sexual stages, as in the case of primaquine; they may directly inhibit gametocyte development by preventing development of the younger sequestered stages (stages I to III); or they may simply prevent gametocytogenesis through rapid elimination of the asexual stages. These results confirm that artesunate is more effective than quinine in the prevention of gametocyte development.

Primaquine at all studied doses showed significant gametocytoicidal effects, in that gametocyte clearance was accelerated, but primaquine did not prevent gametocyte development, and there was no evidence of synergy with quinine or artesunate against asexual stages of *P. falciparum*. The incidences of gametocytemia in patients treated with combined primaquine regimens were not significantly different from those for the nonprimaquine regimens in either the quinine or artesunate groups, but combinations including primaquine shortened the duration of gametocyte carriage two- to sixfold compared to the corresponding regimens without primaquine (P ≤ 0.038). This corresponds with earlier studies and indicates that primaquine is a potent gametocytocidal drug. In the present study, the overall GCTs did not correlate with PCTs, supporting the unrelated activities of primaquine against asexual and mature gametocyte stages of *P. falciparum*. In summary, the results of the present study indicate that artesunate is a potent inhibitor of gametocytegenesis but is inferior to primaquine in terms of gametocyte clearance. In reducing transmission potential, primaquine had a greater effect when added to quinine than to artesunate. Of all the studied regimens, the artesunate-primaquine combination gave the lowest rate of gametocyte detection and the shortest duration of gametocytemia.

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