Efficacy of Primaquine Regimens for Primaquine-Resistant
Plasmodium vivax Malaria in Thailand

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Abstract. To define the current efficacy of Fansidar® (F. Hoffmann-La Roche Ltd., Basel Switzerland) (pyrimethamine and sulfadoxine), primaquine in a high dose, and artesunate for treating acute Plasmodium vivax malaria, we conducted a comparative clinical trial of these 3 drugs in an open-label study. Patients (15–65 years old) were assigned to 1 of 4 treatments regimens in a serial order. Ninety percent of the patients were infected at Thailand-Myanmar border. Patients in group I (n = 23) received Fansidar® (3 tablets, 75 mg of pyrimethamine and 1,500 mg of sulfadoxine, a single dose on the first day), group II (n = 23) received Fansidar® (3 tablets, 75 mg of pyrimethamine and 1,500 mg of sulfadoxine, a single dose on the first day) and then received primaquine (30 mg a day for 14 days), group III (n = 23) received primaquine (30 mg a day for 14 days), and group IV (n = 23) received artesunate (200 mg once a day for 3 days) and then primaquine (30 mg a day for 14 days). Cure rates on day 28 of follow-up were 40%, 100%, 100%, and 100% in groups I, II, II, and IV, respectively. There were 4 and 5 patients in group I showing post-treatment reappearance of parasitemia at ≤ 16 days and between 17 and 28 days, respectively. Patients in the other 3 groups showed negative parasitemias within 7 days after treatment. Artesunate plus primaquine (group IV) cleared parasitemia faster than the other 3 regimens. There is a high proportion of ineffectiveness of Fansidar® for treatment of P. vivax malaria and it should be no longer used for treatment of P. vivax malaria acquired at the Thailand-Myanmar border. A high dose of primaquine is safe and effective in the treatment of P. vivax malaria during the 28-day follow-up period.

Plasmodium vivax causes a clinically benign illness but relapse is frequent. It is a common cause of malaria in Asia and South America. In a recent study among a population residing on the western border of Thailand, P. vivax was found in 2,573 (54%) of 4,728 persons over a period of 2 years.1 Currently, the treatment of choice is a 3-day course of chloroquine in a total dose of 1,500 mg following by primaquine, 15 mg a day for 14 days. This regimen often causes resolution of acute symptoms and clearing of the parasitemia with a significant number of relapses.2,3 In recent years, a number of chloroquine-resistant strains have been reported from many regions in the world.4–8

Fansidar® (F. Hoffmann-La Roche Ltd., Basel Switzerland), a combination of pyrimethamine and sulfadoxine, has been used to treat and prevent malaria for many decades, although resistance in P. falciparum to this drug has developed in many areas. A study in 19799 showed that it was active against P. vivax but less effective than chloroquine. After its use for decades, there are no reports available about its efficacy for treating acute P. vivax malaria.

Primaquine is the only drug available in the market to prevent relapse of P. vivax from hypnozoites in the liver. The efficacy of conventional doses is approximately 80%.10,11 A study conducted at our institute showed improvement of cure rate when the dose was increased.10 The dosage has been limited to a maximum of 30 mg/day because of toxicity. Primaquine has some activity as a blood schizonticide.12

Artesunate, an artemisinin derivative, at a dose 600 mg in over a 3-day period has been used in some regions in Thailand and at our institute as a standard treatment of adults patients with P. falciparum malaria. Artesunate also has shown good activity against P. vivax.13 As resistance to chloroquine continues to develop (and in cases of mixed infection: P. falciparum and P. vivax, or species of Plasmodium that could not be identified), artemisinin derivatives may be a possible alternative treatment in the future.

To define the current efficacy of Fansidar®, primaquine at a high dose and artesunate for treating acute P. vivax malaria, we conducted a clinical trial of these 3 drugs in 4 regimens in an open-label comparison.

Patients and Methods

Patients admitted to the Bangkok Hospital for Tropical Diseases between March 1997 and December 1998 were included into the study if they were diagnosed as having P. vivax malaria. Patients enrolled in the study were 15–65 years old, weighed 40–65 kg, gave informed consent, and agreed to remain in hospital for a total of 28 days. Reasons for exclusion included pregnancy, concomitant infection with P. falciparum at presentation, a history of antimalarial ingestion in the previous 2 weeks, a history of allergy to sulfadoxine or pyrimethamine, and a history of dark urine or significant hemoglobinuria during the course of previous malarial attack. The study protocol was approved by the Ethics Committee of the Mahidol University.

Patients were assigned to 1 of 4 treatment regimens in a serial order. Patients in group I received pyrimethamine, 75 mg, and sulfadoxine, 1,500 mg (Fansidar®, 3 tablets) in a single dose on the first day, group II received pyrimethamine, 75 mg, and sulfadoxine 1,500 mg (Fansidar®, 3 tablets) in a single dose on the first day and then received primaquine, 30 mg once a day for 14 days, group III received primaquine, 30 mg once a day for 14 days, and group IV received artesunate, 200 mg once a day for 3 days and then received primaquine, 30 mg once a day for 14 days.

Oral temperature, pulse, and respiratory rates were measured every 4 hr and blood pressure was measured once a day. Monitoring for signs and symptoms of malaria was per-
formed daily for the first 7 days of admission and weekly thereafter. All of these patients were closely monitored for the clinical of intravascular hemolysis and hemoglobinuria.

Pretreatment investigations included full blood count (red blood cell count, hemoglobin, hematocrit, total white blood cell count, differential count, and platelet count), electrolytes, total and direct bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, albumin, globulin, aspartate and alanine aminotransferases, and urinalysis. These tests were repeated on days 7, 14, 21, and 28. A screening test for glucose-6-phosphate dehydrogenase deficiency was performed on admission. Thick and thin blood films were examined before treatment and every 12 hr for malaria parasites until negative, then thick films were performed daily until discharge. Parasite counts per microliter were determined by counting the number of asexual parasites per 200 white blood cells in thick films or per 1,000 red blood cells in thin films. Blood films were considered negative if no parasite were seen in 200 oil-immersion fields in a thick blood film.

Fever clearance time (FCT) was defined as the time from the start of treatment until the oral temperature decreased to 37.0°C and remained below this for the next 48 hr. Parasite clearance time (PCT) was defined as the time from the start of treatment until the first negative blood film and remained negative for the next 24 hr. Fifty percent or 90% PCTs were defined as the time calculated from the start of treatment until parasitemia decreased by either 50% or 90% of the initial values, respectively. The response to treatment was defined according to Baird and others' as follows: if the reappearance of parasitemia occurred before day 16, it is almost certainly a recrudescence; between days 17 and 28 it may be either a recrudescence or a relapse. Recurrences beyond day 28 could be a relapse.

Patients with a reappearance of parasitemia after treatment with any of the 4 regimens were treated with chloroquine (30 mg/kg) and primaquine (15 mg once a day for 14 days) as a standard treatment of the hospital. The patients with subsequent appearance of a sexual form of *P. falciparum* were treated with either quinine and tetracycline or artesunate, 600 mg, followed by mefloquine, 25 mg/kg. These 6 patients were excluded from the assessment of cure rate at 28 days.

Parasite reductions by the 4 regimens were assessed by the least significant difference method.

**RESULTS**

Ninety-two patients were enrolled in the study, of whom there were 23 in each group. Demographic clinical data and pretreatment laboratory characteristics are shown in Table 1. The 4 groups were comparable at baseline with respect to both clinical and laboratory characteristics. The majority of the patients (90%) were experiencing their first malarial attack and had contracted the infection at the Thailand-Myanmar border.

Following treatment, 15 patients (1 in group I, 3 in group II, 4 in group III, and 7 in group IV) left the hospital before completing 28 days of follow-up (dropped out) (on day 10; days 5, 7, and 20; days 7, 15, 18, and 21; and days 7, 8, 9, 14, 21, and 21 in groups I, II, III, and IV, respectively) due to social reasons unrelated to drug treatment or side effects. All were asymptomatic and negative for asexual forms before discharge from the hospital.

In group I, there were 4 and 5 patients with parasitemia at ≤16 days (median = 12 days, range = 8–16 days) and between 7 and 28 days (median = 21 days, range = 18–28 days) of treatment. Therefore only 6 (40%) of 15 patients were considered cured during the 28-day follow-up period. There were no parasitemias after 7 days of treatment in groups II, II, and IV and the cure rates at day 28 for the patients in these 3 groups were all 100%.

During the follow-up period, 6 (9%) of 66 patients (2 in group I, 1 in group II, 2 in group III, and 1 in group IV) had asexual forms of *P. falciparum* in blood smears. The days of the appearance of *P. falciparum* in each group are shown in Table 2. All 6 patients were successfully treated with either quinine and tetracycline for 7 days or artesunate, 600 mg, followed by mefloquine, 25 mg/kg. These 6 patients were excluded from the assessment of cure rate at 28 days.

The FCT and PCT of each of the 4 groups are shown in Table 2. The patients in the artesunate plus primaquine arm (group IV) had a significantly shorter FCT than the those of the other 3 groups (*P = 0.003*). There were significant differences (*P < 0.001*) in the PCT among them except in the Fansidar® plus primaquine arm (group II) versus the primaquine alone arm (group III). The artesunate plus primaquine arm (group IV) had the shortest PCT. The longest PCT was observed in the Fansidar® arm (group I).

Parasite reductions by the 4 regimens were assessed by the 50% and 90% parasite reduction compared with the initial counts before treatment. These trends are shown in Figure 1. Artesunate plus primaquine cleared the parasitemia faster than any of the other 3 regimens and the difference was statistically significant (*P < 0.001*).

There were no serious adverse effects from primaquine, including hemolysis, cyanosis, abdominal cramp, hypertension, arrhythmias, central nervous system symptoms, granulocytopenia, agranulocytosis, or leukocytosis, in groups II, III, and IV. Two patients were found to be deficient for glucose-6-phosphate dehydrogenase, 1 in group I and 1 in group III. However, only 1 patient in group III had a significant decrease in his hematocrit, possibly due to hemolysis. His hematocrit decreased from 32% before treatment to 21% on day 5 after 4 days of treatment (4 doses) of primaquine (30 mg once a day). There was no clinical evidence of dark urine and a diagnosis of hemolysis was not confirmed by our laboratory. The drug was withheld for 3 days and a transfusion with a unit of packed red blood cells was given. Thereafter, his hematocrit increased to 31% and primaquine (30 mg once a day) was again given to complete the treatment course (14 days). He remained well during the 28-day follow-up interval. All patients in groups I, II, and IV tolerated this high dose of primaquine during the 28-day follow-up period.
Efficacy of Primaquine for *P. vivax* Malaria

**TABLE 1**
Clinical and laboratory characteristics of the 4 study groups before treatment*

<table>
<thead>
<tr>
<th>Group</th>
<th>Male/female (n = 23)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Fever [mean (±SD)]</th>
<th>Number with (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13/10</td>
<td>23.5 (6.9)</td>
<td>15–41</td>
<td>159.0 (7.0)</td>
<td>5.3 (3.0)</td>
<td>48.9 (6.0)</td>
</tr>
<tr>
<td>II</td>
<td>21/2</td>
<td>24.1 (8.7)</td>
<td>16–52</td>
<td>163.0 (8.0)</td>
<td>6.0 (4.0)</td>
<td>52.1 (9.4)</td>
</tr>
<tr>
<td>III</td>
<td>18/4</td>
<td>20.4 (6.4)</td>
<td>15–40</td>
<td>160.7 (8.4)</td>
<td>5.7 (3.8)</td>
<td>51.0 (8.7)</td>
</tr>
<tr>
<td>IV</td>
<td>16/7</td>
<td>24.5 (7.5)</td>
<td>15–46</td>
<td>160.2 (7.8)</td>
<td>5.2 (3.0)</td>
<td>52.2 (7.0)</td>
</tr>
</tbody>
</table>

Laboratory data [mean (±SD)]

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 23)</th>
<th>Group II (n = 23)</th>
<th>Group III (n = 23)</th>
<th>Group IV (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume (%)</td>
<td>35.9 (7.1)</td>
<td>37.7 (8.4)</td>
<td>35.3 (6.2)</td>
<td>35.2 (6.8)</td>
</tr>
<tr>
<td>WBC count (per µl)</td>
<td>7,087 (2,342)</td>
<td>6,257 (2,107)</td>
<td>6,339 (2,619)</td>
<td>7,113 (3,557)</td>
</tr>
<tr>
<td>BUN (mg%)</td>
<td>15.9 (5.2)</td>
<td>17.7 (4.3)</td>
<td>14.8 (4.0)</td>
<td>14.8 (4.3)</td>
</tr>
<tr>
<td>Creatinine (mg%)</td>
<td>0.97 (0.21)</td>
<td>1.07 (0.22)</td>
<td>0.93 (0.13)</td>
<td>0.96 (0.16)</td>
</tr>
<tr>
<td>Total bilirubin (mg%)</td>
<td>1.7 (1.1)</td>
<td>1.5 (0.8)</td>
<td>1.4 (1.2)</td>
<td>1.6 (1.6)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>39.6 (30.3)</td>
<td>35.7 (22.4)</td>
<td>34.7 (33.8)</td>
<td>34.5 (19.8)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>36.5 (46.1)</td>
<td>27.2 (20.1)</td>
<td>34.4 (51.2)</td>
<td>31.5 (35.2)</td>
</tr>
<tr>
<td>Albumin (mg%)</td>
<td>3.9 (0.5)</td>
<td>4.1 (0.5)</td>
<td>3.9 (0.5)</td>
<td>3.9 (0.5)</td>
</tr>
</tbody>
</table>

* G-6-PD = glucose-6-phosphate dehydrogenase; WBC = white blood cell; BUN = blood urea nitrogen; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

**DISCUSSION**

The treatment of malaria caused by *P. vivax* has 2 objectives: to cure the clinical symptoms and to prevent their relapse. For the first objective, chloroquine has been the standard treatment for the last 40 years, although chloroquine-resistant strains have spread over the past several decades.1,14 However, resistance of *P. vivax* to chloroquine has been recognized.15 For *P. falciparum* to Fansidar®, a combination of pyrimethamine and sulfadoxine, was introduced to either treat or prevent malaria several decades ago.1–8 Resistance of *P. vivax* to Fansidar® is well recognized.9–11 For *P. vivax*, this picture is unclear. Our study demonstrates that *P. vivax* shows a high proportion of resistance to Fansidar® at the present time. We suggest that Fansidar® no longer be used in the treatment of even mild illness caused by *P. vivax*.

Primaquine has been used for preventing relapse due to hypnozoites in the liver. The conventional dose is 15 mg/day for 14 days. This regimen was reported to be 82% effective.16 Increasing the daily dose to 22.5 mg/day improved the cure rate to 97%.17 However, the dosage was limited to ±30 mg/day due to its toxicity. Although it was reported that the blood schizonticidal effect of primaquine would not be achieved unless used in toxic doses,12 a recent study showed clinical efficacy in the treatment of *P. vivax* malaria.12 The patients in group III treated with high-dose primaquine alone were all cured at day 28. All patients in group II were also cured. When compared with the result in group I, the results in groups II and III with the 100% cure rates were due to co-administration of primaquine.

Post-treatment reappearance of parasitemia may arise from reinfection, relapse, or recrudescence. The study patients residing in Bangkok precluded reinfection as a possible source of post-Fansidar® reappearance of parasitemia because there are no reported cases of human malaria in this city. Baird and others14 had studied the timing or recurrence of *P. vivax* infection in hundreds of tropical Asian strains of *P. vivax* and found that parasitemia by *P. vivax* recurring in the 28-day period after full compliance with a standard chloroquine therapy demonstrates resistance. If the recurrence appears before day 16, it is almost certainly a recrudescence and if it appears between days 17 and 28, it may be either a recrudescence or a relapse by chloroquine-resistant parasites. Recurrences beyond day 28 could be a relapse by chloroquine-sensitive *P. vivax*. In Table 2, 4 patients who had a reappearance of parasitemia at ≤16 days (median = 12 days, range = 8–16 days) should be recrudescences and 5 patients who had parasitemias between 17 and 28 days (median = 21 days, range = 18–28 days) represented either recrudescences or relapses. Since we did not follow-up the patients longer than 28 days in this study, we do not know how many patients relapsed after day 28. However, mixed broods of naturally acquired *P. vivax* may cause relapse at almost any point after therapy.
Artesunate, an artemisinin derivative, has been widely accepted for the treatment of *P. falciparum* malaria in areas of multidrug-resistant *P. falciparum*. Our study shows that the immediate response of a short course (3 days) of single daily doses of artesunate for treatment of *P. vivax* malaria is similar to the immediate response obtained with chloroquine treatment of *P. vivax* malaria in our previous study. Rapid reduction in fever and the number of parasites were observed in *P. vivax*-infected patients treated with artesunate, similar to that in *P. falciparum*-infected patients in our previous study. Table 2 shows that group IV patients had the most rapid PCT compared with other 3 groups. In group IV, primaquine was sequentially given at 72 hr, in which the mean ± SD PCT was 41.1 ± 14.3 hr (range = 6–67 hr). Thus, the rapid PCT in group IV patients is likely due to treatment with artesunate.

One patient in group IV developed *P. falciparum* malaria during the follow-up period despite the activity of artesunate for *P. falciparum*, and occurrences of *P. falciparum* malaria were similar to those in the other groups. The results in groups II, III, and IV with high-dose primaquine treatment show that neither Fansidar® nor artesunate increase the cure rate at day 28 because high-dose primaquine alone already shows a cure rate of 100%. The benefit of co-administration of artesunate is rapid parasite clearance after starting treatment.

Six (6%) of 66 evaluable patients with *P. vivax* infections had asexual forms of *P. falciparum* during the 28-day period after the initial treatment. This was similar in all 4 groups. This implies that mixed infections of *P. falciparum* and *P. vivax* were present on admission. We previously reported that one-third of patients with *P. falciparum* malaria had *P. vivax* malaria within the 2-month follow-up period. This result should warn clinicians of the importance of follow-up (for possible relapse, recrudescence, or mixed infections) before patients are discharged.

In conclusion, there is a high proportion of ineffectiveness of Fansidar® for treatment of *P. vivax* malaria, and it should no longer be used in the treatment of *P. vivax* malaria acquired on the Thailand-Myanmar border. A high dose of primaquine is safe and effective in producing clinical and parasitic cure of *P. vivax* malaria during the 28-day follow-up period. Further in-depth studies to define the role of this drug
is needed. Thus, primaquine may be considered as an alternative regimen for the treatment of \textit{P. vivax} malaria.

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