Plasma Concentrations of Tafenoquine, a New Long-Acting Antimalarial Agent, in Thai Soldiers Receiving Monthly Prophylaxis

Michael D. Edstein,1 David A. Kocisko,1 Douglas S. Walsh,2 Chirapa Eamsila,2 Bruce G. Charles,2 and Karl H. Rieckmann1

1Australian Army Malaria Institute and 2Australian Centre for Paediatric Pharmacokinetics, School of Pharmacy, University of Queensland, Brisbane, Australia; and 3US Army and Thai Medical Components, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

We measured plasma tafenoquine concentrations in Thai soldiers given a monthly regimen of tafenoquine to determine whether these concentrations adequately suppressed malarial infections on the Thai-Cambodian border. After receiving a treatment course of artesunate and doxycycline, 104 male soldiers were administered a loading dose of tafenoquine (400 mg daily for 3 days), followed by tafenoquine monthly (400 mg every 4 weeks) for 5 months. Consecutive monthly mean (± standard deviation) trough plasma tafenoquine concentrations were 223 ± 41, 127 ± 29, 157 ± 51, 120 ± 24, and 88 ± 20 ng/mL. Only 1 soldier developed malaria during the study. At the time of malaria diagnosis, his plasma tafenoquine concentration was 40 ng/mL, which was ~3-fold lower than the trough concentrations of the other soldiers. Although low tafenoquine concentrations appear to be uncommon, additional investigations are needed to determine the relationship between plasma tafenoquine concentrations and suppression of malaria.

The choice of drugs for malaria prophylaxis is limited because of the continuing spread of multidrug-resistant Plasmodium falciparum malaria and adverse reactions associated with antimalarial drugs. The limited options available for malaria prophylaxis has stimulated the development of new antimalarial drugs. Tafenoquine (8-[4-amino-1-methylbutyl]amino]-2,6-dimethoxy-4-methyl-5-[3-trifluoromethyl-phenoxy] quinoline, succinate), also known as WR 238605, is a new 8-aminooquinoline antimalarial drug being jointly developed by the Walter Reed Army Institute of Research and GlaxoSmithKline Pharmaceuticals as a potential replacement for primaquine.

Tafenoquine is well tolerated after oral administration to volunteers, with only mild and transient gastrointestinal effects [1]. In 1997, tafenoquine was given weekly at a dose of 200 mg or 400 mg base to black adult Kenyans in an area in western Kenya where P. falciparum is holoendemic and was shown to be highly effective and well tolerated [2]. As part of this trial, a 3-day treatment regimen of 400 mg of tafenoquine per day, followed by weekly doses of placebo, protected 82% (95% CI, 59%–92%) of participants for 7 weeks after commencement of drug administration. The prophylactic value of tafenoquine was further demonstrated in clinical studies in Ghana and Gabon, with protective efficacies of >86% using lower-dose regimens of 200 mg per week [3] or 200 mg per day for 3 days [4].

The minimum effective plasma drug concentration for malaria prophylaxis is difficult to determine, because genetic factors, drug absorption, immunity status, and varying susceptibility of malaria parasites to drugs.
can markedly affect the relationship between drug concentration and efficacy. In contrast to primaquine, the therapeutic index of tafenoquine is greater and, consequently, higher doses of tafenoquine can be safely administered to individuals. The recommended maximum daily adult dose of primaquine for prophylaxis is 30 mg base [5]. Peak plasma primaquine concentrations of $\sim$80 ng/mL occur 2 h after dosing, with trough concentrations (24 h after dosing) of $\sim$10 ng/mL [6]. For tafenoquine, peak blood concentrations of 417–489 ng/mL are observed after administration of a dose of 600 mg to healthy volunteers, with a half-life of $\sim$2 weeks [7]. In view of these pharmacological characteristics and the prolonged suppression of parasitemia after receiving a 3-day loading dose (total, 1200 mg base) of tafenoquine in the Kenya study [1], it was believed that administration of tafenoquine at only monthly intervals might still provide an acceptably high level of protection against malaria.

This led to a field study in which Thai soldiers received tafenoquine during their deployment to the border of Thailand and Cambodia, an area where *P. falciparum* and *Plasmodium vivax* are endemic. The soldiers were given a loading dose of 1200 mg of tafenoquine (400 mg q.d. for 3 days), followed by 400 mg once per month for 5 months. On the basis of earlier observations [1, 2], it was anticipated that monthly plasma trough tafenoquine concentrations would not decrease to less than 80–100 ng/mL during a period of 5–6 months and that it would not be unreasonable to expect such trough tafenoquine concentrations to suppress malaria.

**VOLUNTEERS AND METHODS**

**Study population, design, and dosing.** In this study, 205 male Thai soldiers deployed on security operations along the Thai-Cambodian border in the district of Nam Yun, Ubol Ratchatani province, volunteered to participate in a double-blind, randomized, placebo-controlled phase II clinical trial to determine the effectiveness and safety of tafenoquine for malaria prophylaxis. The findings of the clinical study will be published elsewhere. The study was approved by the US Army Human Use Research Review Board and the institutional review boards of the Royal Thai Army, the Thai Ministry of Public Health, and the Thai Food and Drug Administration. Informed consent was obtained from all volunteers. The volunteers, who were glucose-6-phosphate dehydrogenase normal, were judged to be healthy on the basis of physical examination and had normal biochemical and hematological indices.

All volunteers were presumptively treated with artesunate (300 mg on day 0, followed by 150 mg q.d. on days 1 and 2) plus 200 mg of doxycycline per day for 7 days to cure any preexisting malaria infection. After receiving the treatment regimen, 104 soldiers (drug group) received a loading dose of 400 mg base of tafenoquine per day for 3 days, followed by 400 mg of tafenoquine per month (once every 4 weeks) for 5 consecutive months. In the placebo group (101 soldiers), 30 soldiers developed malaria during the study period. They were retreated with artesunate-doxycycline, and all of them opted to receive a loading dose of 400 mg of tafenoquine per day for 3 days, followed by 400 mg of tafenoquine at weekly intervals. Tafenoquine (as the succinate salt) was provided by SmithKline Beecham in unmarked gelatin capsules containing 250 mg of salt (equivalent to a 200-mg base), and was swallowed with water (80–100 mL) and food (cake and biscuits).

**Blood sampling.** Blood samples were obtained for drug analysis randomly in the field from volunteers receiving the monthly tafenoquine regimen and from soldiers who, after developing malaria, decided to take weekly doses of tafenoquine. During the 7-month study (6 months of chemosuppression and 1 month of follow-up), samples were obtained $\sim$8 h, $\sim$24 h, $\sim$48 h, and $\sim$56 h after commencing the 3-day loading dose and then at 3- to 4-day intervals thereafter until administration of the first monthly dose. After each monthly dose, samples were obtained at $\sim$8 h after receiving the dose, mid-month, and at the end of the month (trough level). Samples were obtained $\sim$4 h, $\sim$8 h, $\sim$12 h, and $\sim$24 h after receiving the last monthly dose and at 3- to 4-day intervals thereafter for 2 months. The maximum number of blood samples obtained each month from a volunteer was 3 samples, with at least a 2-day break between each collection. At each blood collection, samples were obtained from 1–27 volunteers (median, 13; mean ± SD, 12.6 ± 6.7). Blood samples were also obtained from volunteers who received weekly doses of tafenoquine $\sim$12 h after drug administration and immediately before the next dose.

Blood samples (7 mL) were transferred into EDTA tubes and transported on ice to the field laboratory within 1 h after collection. Whole blood samples were centrifuged at $\sim$1200 g for 15 min, and the separated plasma was stored on dry ice for up to 1 week pending transport to the Armed Forces Research Institute of Medical Sciences (Bangkok, Thailand) for storage at $\sim$70°C. The samples were then air freighted on dry ice to the Army Malaria Institute (Brisbane, Australia) for storage at $\sim$70°C until analysis.

**Drug analysis.** Tafenoquine concentrations in plasma were measured by reversed-phase high-performance liquid chromatography, with fluorescence detection, as described elsewhere [8]. The interday and intraday coefficients of variation (assay range, 20–1000 ng/mL) were $\leq$8.4%, with a mean inaccuracy of 7.3%. The lower limit of quantification was 10 ng/mL.

**Monitoring for malaria infection.** Thick and thin smears were prepared from finger-prick samples of blood and stained with Giemsa stain. They were obtained when the volunteers were thought to have malaria and on any day a volunteer was ill. The blood smears were examined independently for malaria
parasites by 2 microscopists. If there was a disagreement between the 2 microscopists, the senior technologist present would adjudicate. Parasite density was determined by counting the number of asexual parasites per 200 WBCs and multiplying by 40 to obtain the approximate number of parasites per microliter of blood. A blood slide was not considered negative until an examination of 200 oil immersion thick fields (magnification, ×1000) showed no parasites. The first blood smear confirmed positive for malaria was considered to indicate a failure of prophylaxis.

RESULTS

The mean age and weight of the volunteers who received tafenoquine were 29 years (median, 23 years; range, 21–46 years) and 59.6 kg (median, 59.5 kg; range, 45–80 kg), respectively. The mean plasma concentration-time profile (± SD) of tafenoquine after receiving the loading dose (400 mg q.d. for 3 days) and monthly 400-mg doses is presented in figure 1. Interindividual plasma tafenoquine concentrations at various times after commencement of the tafenoquine loading dose were large.

Table 1 shows mean plasma tafenoquine concentrations during the loading-dose phase. In accordance with published findings, tafenoquine exhibits linear pharmacokinetics [1], with plasma values increasing proportionally with dose during the loading phase. Minimum steady-state tafenoquine concentrations of 80–100 ng/mL (table 2) were observed with the monthly 400-mg regimen. As with the loading dose, large interindividual variability in tafenoquine concentrations was also observed at the end of each monthly dosing period.

Only 1 volunteer receiving the tafenoquine regimen developed malaria (P. vivax load, 1472 parasites/μL of blood). In contrast to the volunteers who did not develop parasitemia while receiving tafenoquine, this volunteer had a plasma tafenoquine concentration of 40 ng/mL at the time of malaria diagnosis. This was about 3-fold lower than that for the volunteers who did not develop parasitemia. He also had consistently lower tafenoquine concentrations on 6 earlier occasions after receiving the loading dose, compared with the mean tafenoquine values of the soldiers who did not develop parasitemia (figure 1).

In the 30 soldiers of the placebo group (n = 101) who developed malaria during the study and who received weekly doses of tafenoquine (400 mg), mean steady-state weekly plasma tafenoquine concentrations (± SD) ranged from 612 ± 115 ng/mL (range, 416–900 ng/mL) in 20 soldiers at ~12 h after dosing to 441 ± 76 ng/mL (range, 263–581 ng/mL) in 28 soldiers immediately before administration of the next dose. None of the volunteers receiving weekly doses of tafenoquine developed malaria during the study period. The soldier for whom monthly tafenoquine prophylaxis failed showed a continuing trend of relatively lower plasma tafenoquine concentrations after weekly prophylaxis, with plasma tafenoquine concentrations ranging from 395–458 ng/mL (n = 2) at ~12 h after dosing to 236–301 ng/mL (n = 3) immediately before receiving the next weekly dose.

After completion of monthly tafenoquine prophylaxis, 5 soldiers developed malaria 6–12 weeks after receiving the last monthly dose. Plasma tafenoquine concentrations could be measured in 3 of 5 soldiers at the time of malaria diagnosis. Two of the soldiers were infected with P. vivax malaria; their tafenoquine concentrations were 20 ng/mL and 21 ng/mL. The other soldier had P. falciparum malaria, with a tafenoquine concentration of 38 ng/mL.

DISCUSSION

This study showed that after receiving a loading dose of tafenoquine (total dose, 1200 mg), followed by monthly doses of 400 mg, minimum steady-state plasma concentrations of tafenoquine of 80–100 ng/mL were achieved for ≥5 months. This regimen provided a protective efficacy for tafenoquine of 97% (95% CI, 82%–99%) against both falciparum and vivax malaria in an area where multidrug-resistant P. falciparum is endemic.
The drug was well tolerated by the volunteers, with only mild and transient side effects (headache and diarrhea) reported.

When optimizing tafenoquine regimens for malaria prophylaxis, information concerning minimum tafenoquine concentrations that suppress malaria becomes rather important. In this study, we have shown that plasma tafenoquine concentrations of <40 ng/mL are unable to suppress asexual parasites in an area on the border of Thailand and Cambodia where both *P. falciparum* and *P. vivax* are endemic. On the basis of these findings, it is recommended that future tafenoquine prophylaxis studies aim to achieve plasma tafenoquine concentrations of >80 ng/mL to provide at least a 2-fold margin of protection. This can be achieved by increasing either the dose or the frequency of tafenoquine administration.

We showed that the population pharmacokinetics of tafenoquine in the Thai soldiers were in accordance with those in other studies, with a mean plasma clearance of 3.20 L/h and an elimination half-life of 16.4 days [10]. Of the 101 soldiers who received tafenoquine, only 1 soldier developed malaria. He had vivax malaria diagnosed 5 weeks after receiving the second monthly dose. He was not administered his third monthly dose because he was away from the study area, on leave. Nevertheless, when malaria was diagnosed, his tafenoquine concentration of 40 ng/mL was ~3 times lower than that of soldiers measured at 4 weeks after receiving the second monthly dose. From the population analysis, his estimated clearance of tafenoquine was considerably greater than that of the soldiers who did not develop parasitemia (5.46 vs. 3.20 L/h), and his elimination half-life of tafenoquine was markedly shorter (10.7 vs. 16.4 days) [10]. His apparent volume of distribution for tafenoquine (2018 L) was similar to the mean value for the soldiers who did not develop parasitemia (1820 L; 95% CI, 1360–2430 L). This soldier also had lower tafenoquine concentrations when he was subsequently given weekly 400-mg doses of tafenoquine. It is unclear why he had lower tafenoquine concentrations while receiving either weekly or monthly prophylaxis. His age (22 years) and weight (63 kg) were similar to the median values (23 years and 59.5 kg) for the soldiers receiving monthly doses of tafenoquine, and, on the basis of hematological and biochemical indices, he was judged to be healthy, with no prior history of disease.

Because antimalarial activity is related to plasma drug concentrations for effective prophylaxis, it makes sense to investigate various factors that might affect drug concentrations and that may explain why some individuals have low tafenoquine concentrations. Such factors as food and metabolism have been shown to affect drug concentrations of several antimalarial drugs in the same ethnic group. It is well known that the coadministration of food can affect the absorption and bioavailability of atovaquone (Malarone [GlaxoSmithKline], a fixed combination of atovaquone and proguanil) [11] and mefloquine [12], resulting in a wide range of plasma drug concentrations. Tafenoquine is also affected by food: a standard high-fat meal increases oral bioavailability by ~40% (A. K. Miller, personal communication). Population kinetics of tafenoquine in the Thai soldiers revealed that the amount of tafenoquine absorbed, rather than the rate of absorption, is likely to be most affected by food [10]. In the present study, however, it is unlikely that food was a contributing factor towards the low tafenoquine concentrations observed in the soldier who developed malaria, because he consumed the same small meal

### Table 1. Plasma tafenoquine concentrations after commencing administration of the loading dose (400 mg q.d. for 3 days).

<table>
<thead>
<tr>
<th>Time that blood sample was obtained</th>
<th>No. of volunteers</th>
<th>Tafenoquine concentration, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–8 h after dosing</td>
<td>22</td>
<td>210</td>
</tr>
<tr>
<td>24 h after dosing</td>
<td>26</td>
<td>251</td>
</tr>
<tr>
<td>Day 2 at 24 h after dosing</td>
<td>18</td>
<td>492</td>
</tr>
<tr>
<td>Day 3 at 7–8 h after dosing</td>
<td>18</td>
<td>730</td>
</tr>
</tbody>
</table>

### Table 2. Monthly trough plasma concentrations of tafenoquine.

<table>
<thead>
<tr>
<th>Time that blood sample was obtained</th>
<th>No. of volunteers</th>
<th>Trough tafenoquine concentration, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>At the end of the first month</td>
<td>17</td>
<td>238</td>
</tr>
<tr>
<td>At the end of the second month</td>
<td>11</td>
<td>124</td>
</tr>
<tr>
<td>At the end of the third month</td>
<td>14</td>
<td>145</td>
</tr>
<tr>
<td>At the end of the fourth month</td>
<td>4</td>
<td>117</td>
</tr>
<tr>
<td>At the end of the fifth month</td>
<td>10</td>
<td>86</td>
</tr>
<tr>
<td>At the end of the sixth month</td>
<td>11</td>
<td>101</td>
</tr>
</tbody>
</table>
with tafenoquine as the other soldiers who did not develop parasitemia.

Genetic polymorphism causing individual differences in drug concentrations has been reported for several antimalarial drugs. For instance, individuals are phenotyped as rapid and slow acetylators for dapsone [13] or extensive and poor metabolizers for proguanil [14]. Although preclinical findings have shown that tafenoquine is extensively metabolized and primarily eliminated via biliary excretion [1], no data from studies involving humans have yet been published. Similar to primaquine, the metabolism of tafenoquine is difficult to study, because its structure contains several metabolically labile constituent groups, and its intermediates are unstable and possess amphoteric properties [15]. Thus, the relationship between tafenoquine metabolism and drug concentrations remains to be elucidated.

In conclusion, the rationale for selecting a monthly tafenoquine prophylactic regimen was confirmed, with the measurement of plasma tafenoquine concentrations within the predicted trough concentration range of 80–100 ng/mL. This minimum tafenoquine concentration provided chemosuppression of both falciparum and vivax malaria on the Thai-Cambodian border, whereas concentrations of \( \leq 40 \) ng/mL were unable to do so. Additional studies are required to better characterize the large interindividual differences in plasma tafenoquine concentrations, because some individuals may require dose modification to accommodate intrinsically lower drug concentrations.

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