

Plasmodium vivax Susceptibility to Ferroquine^{∇†}

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The novel organometallic chloroquine analog ferroquine (SSR 97193) is effective against chloroquine-resistant *Plasmodium falciparum*. The *ex vivo* efficacy of ferroquine against *Plasmodium vivax* isolates was tested. Ferroquine has a potent *ex vivo* effect on *P. vivax* schizont maturation (median 50% inhibitory concentration, 15 nM; *n* = 42). No significant cross-sensitivity between ferroquine and other antimalarials was detected. This drug may be a suitable replacement for chloroquine in the treatment of drug-resistant *P. vivax* malaria.

Plasmodium vivax malaria is an underrecognized cause of morbidity in tropical countries outside of Africa. The emergence of resistance to antimalarials and severe forms of the disease *P. vivax* malaria poses a significant global health threat (1, 3). Despite the spread of resistance, chloroquine (CQ) remains the most common first-line treatment for *P. vivax* malaria in pregnant and nonpregnant individuals, being inexpensive and relatively safe. Additionally, the extended half-life of CQ (~30 days) suppresses the first relapse. Although CQ-resistant (CQR) *P. vivax* is generally sensitive to artemisinin therapy, the rapid elimination of artemisinin compounds will prevent relapses, a common feature of *P. vivax* malaria.

Ferroquine (FQ) (SR97193; ferrocene CQ: 7-chloro-4-[[2-(*N,N*-dimethylaminomethyl)]-*N*-methylferrocenylamino}quinoline) a novel 4-aminoquinoline, is thought to have a metabolic profile similar to that of CQ (7) and possess antimalarial activity in the low nanomolar range when tested against CQR *P. falciparum* clones (2, 6, 9) and multidrug-resistant isolates of *P. falciparum* from the Thailand-Myanmar border (4). However, it is not known whether *P. vivax* is sensitive to FQ. This study aimed to examine the effect of FQ (SAR97193) and its demethylated metabolite (FQM; SR97213) on the schizont maturation of clinical isolates of *P. vivax*.

One hundred ten *P. vivax* isolates were collected from October 2006 to April 2009 in the Mae Sot region of Tak Province in northwestern Thailand. All samples were collected from patients with acute *P. vivax* malaria (with a monospecies parasitemia of >100/500 white blood cells) attending the clinics of the Shoklo Malaria Research Unit (SMRU). After written consent was obtained, blood samples were collected by venipuncture in 5-ml lithium-heparinized tubes and arrived at the culture lab at SMRU within 5 h of collection at room temperature. Samples with more than 80% early trophozoites present were chosen for drug sensitivity testing. After platelets and

leukocytes were removed from the isolates (17), *P. vivax* sensitivity was tested as previously described (14, 15). Stage-specific drug activity assays were carried out as previously described (16). The stage specificity experiment was conducted for CQ and FQ in duplicate wells on 10 isolates of *P. vivax* and *P. falciparum*. Plates were quality controlled by testing with a strain of *P. falciparum* (K1) with a known sensitivity profile.

Dose-response curves and IC₅₀ (50% inhibitory concentration) values were calculated by fitting the data to a sigmoidal inhibitory E-max pharmacodynamic model using WINNONLIN Ver 4.1 (Pharsight Corporation). Curves yielding results with a percent coefficient of variation [(standard error × 100)/mean] of <30 were included for further analysis. Final nM IC₅₀s were determined from the salt concentration of the drug. The median IC₅₀ values presented in Fig. 1 were compared using the Kruskal-Wallis test. The Friedman test was used to compare the paired median IC₅₀ values presented in Fig. 2. For post hoc analysis of the data in both Fig. 1 and 2, Dunn's multiple comparison was used. Spearman analysis was used to determine if significant correlations between the sensitivities of the antimalarials tested exist. Statistical analysis and preparation of graphics were carried out using GraphPad Prism 5 software (version 5).

The mean parasitemia of the *P. vivax* isolates tested was 4,432 (range, 1,920 to 7,424) parasites/μl. Of the 110 *P. vivax* isolates tested, 99 were successfully cultured; however, only 63 could be successfully modeled using the strict criteria outlined in the methodology.

P. vivax was significantly more sensitive to artesunate (median IC₅₀, 1.7 nM; 75% confidence interval [CI], 0.6 to 3.4 nM; *n* = 39) than any of the other antimalarials tested (*P* < 0.001) (Fig. 2). FQ (median IC₅₀, 15 nM; 75% CI, 12 to 20 nM; *n* = 52) proved to be as effective as the other antimalarials tested, i.e., CQ (median IC₅₀, 26 nM; 75% CI, 11 to 43 nM; *n* = 63), mefloquine (median IC₅₀, 15 nM; 75% CI, 4 to 47 nM; *n* = 40), and piperazine (median IC₅₀, 32 nM; 75% CI, 22 to 46 nM; *n* = 38) (*P* > 0.05) (Fig. 2). However, *P. vivax* was significantly less sensitive to the FQ metabolite SR97213 (median IC₅₀, 77 nM; 75% CI, 14 to 205 nM; *n* = 20) than to the FQ parent compound (*P* < 0.05) (Fig. 2). FQM has been shown to exhibit

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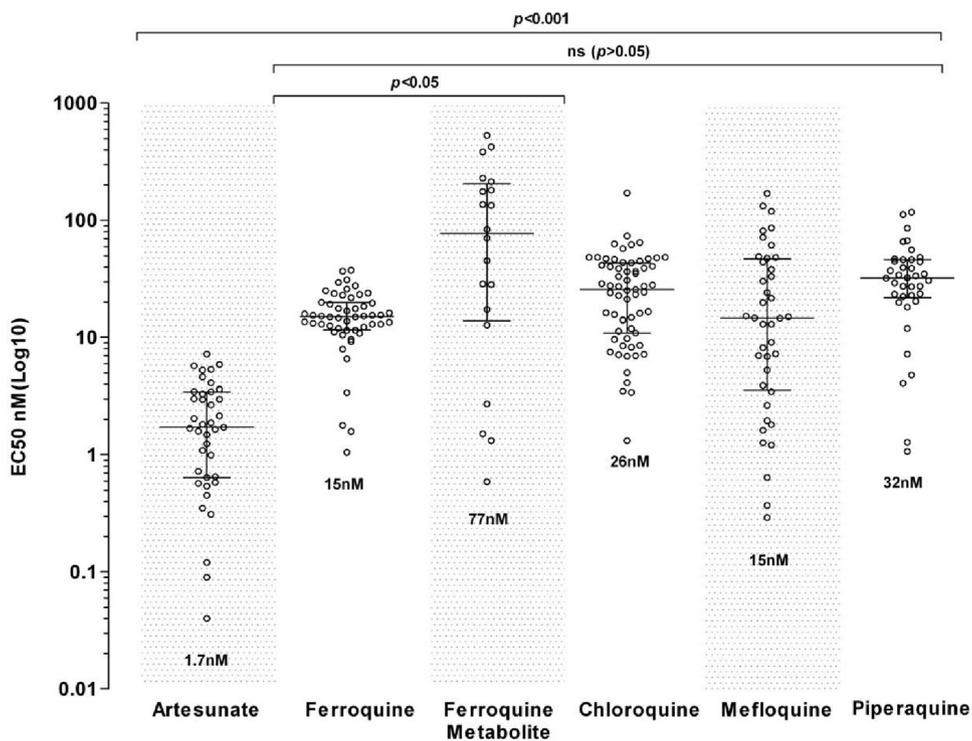


FIG. 1. Median *ex vivo* sensitivities (75% CI) of fresh *P. vivax* isolates from the Thailand-Myanmar border to FQ, its major metabolite, and a range of standard antimalarials. EC50, 50% effective concentration; ns, not statistically significant.

a structure-activity relationship similar to that of the parent compound against *Plasmodium falciparum* (5). Despite some reduction in activity, all FQ metabolites remained more active than CQ against CQR strain Dd2. Further examination of metabolic activity using *in vivo* models demonstrated that despite similar enzymatic targets in the liver (CYP3A4 of the P450 family), the metabolism rate is low (7). Except for a weak significant negative correlation between the sensitivities of *P.*

vivax to CQ and mefloquine (Spearman's $r = -0.4$; $P = 0.02$), no other cross-sensitivity correlations were detected for the other antimalarials tested.

Compared to the ring stages, the mature trophozoites of *P. vivax* and *P. falciparum* were less sensitive to FQ ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 2). However, this stage-specific effect was not as pronounced as the effect of CQ on *P. vivax*, where the IC₅₀ of CQ against mature tro-

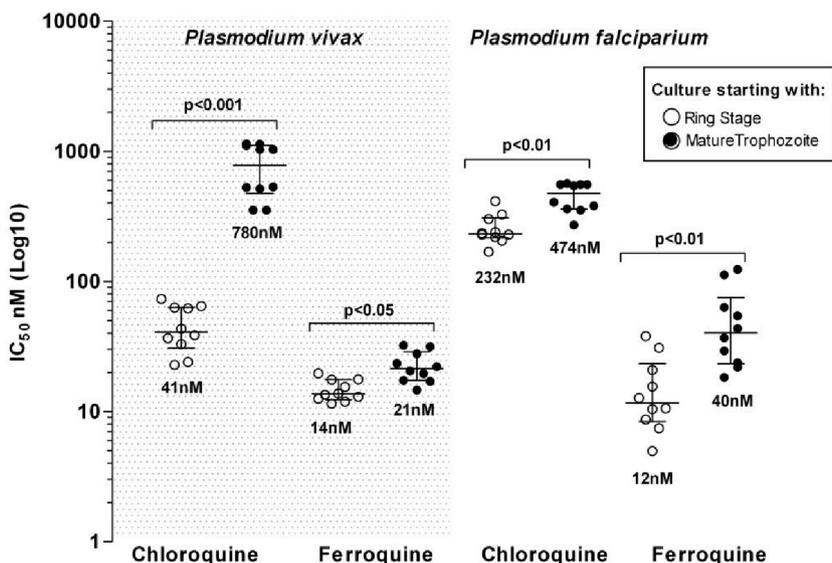


FIG. 2. Comparative stage-specific effects of CQ and FQ on the schizont maturation of *P. vivax* and *P. falciparum* treated at the ring stage versus the trophozoite stage.

phozoites was ~10-fold greater than that against the ring stage ($P < 0.001$) (Fig. 2).

Until recently, it was assumed that *P. vivax* populations on the Thailand-Myanmar border were universally sensitive to non-antifolate antimalarials. However, a number of *ex vivo* studies on *P. vivax* in this region have reported an increased number of isolates with significantly reduced antimalarial sensitivity to CQ and mefloquine (8, 10). Although our data show that most isolates are relatively sensitive to CQ, 1 of the 65 isolates tested for CQ had an IC_{50} of 170 nM. We have also noted an increasing number of patients with *P. vivax* malaria who have experienced early parasitological recurrence (14 to 21 days) after CQ treatment (Aung Pyae Phy, presented at the ASTMH 59th Annual Meeting, Washington, DC, 18 to 22 November 2009).

Our study clearly shows that freshly isolated *P. vivax* and *P. falciparum* are sensitive to FQ at low nM concentrations. Importantly, *P. vivax* isolates were susceptible to FQ, irrespective of the isolates' profiles of sensitivity to CQ and other standard antimalarials. The inability to detect significant FQ cross-sensitivity is in agreement with earlier studies on *P. falciparum* clones. Furthermore, the *P. falciparum* isolates we tested had FQ IC_{50} s similar to that of the W2 strain (6), despite the fact that the Thai isolates were almost 2-fold less sensitive to CQ than this highly CQR clone. This finding confirms the utility of FQ against highly CQR *P. falciparum*.

Although *P. vivax* and *P. falciparum* ring stages were almost twice as sensitive to FQ than were mature trophozoites, this seeming stage specificity is insignificant compared to the stage specificity seen in *P. vivax*. Our data show *P. vivax* trophozoites to be almost 20-fold less sensitive to CQ than the ring stages. This finding confirms earlier work showing that *P. vivax* trophozoites are not susceptible to CQ (12, 14, 16).

Our finding that *P. vivax* is as susceptible to FQ as is CQR *P. falciparum* supports the further development of this schizonticide as a lead antimalarial compound. It would be useful to repeat this *ex vivo* drug sensitivity study in an area of established CQ resistance such as West Papua, Indonesia, where CQ fails to treat a significant proportion of the *P. vivax* malaria cases (11, 13). We anticipate that FQ may be a suitable replacement for CQ in the treatment of drug-resistant *P. vivax* malaria.

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