Open-Label Crossover Study of Primaquine and Dihydroartemisinin-Piperaquine Pharmacokinetics in Healthy Adult Thai Subjects

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Dihydroartemisinin-piperaquine is an artemisinin-based combination treatment (ACT) recommended by the WHO for uncomplicated Plasmodium falciparum malaria, and it is being used increasingly for resistant vivax malaria where combination with primaquine is required for radical cure. The WHO recently reinforced its recommendations to add a single dose of primaquine to ACTs to reduce P. falciparum transmission in low-transmission settings. The pharmacokinetics of primaquine and dihydroartemisinin-piperaquine were evaluated in 16 healthy Thai adult volunteers in a randomized crossover study. Volunteers were randomized to two groups of three sequential hospital admissions to receive 30 mg (base) primaquine, 3 tablets of dihydroartemisinin-piperaquine (120/960 mg), and the drugs together at the same doses. Blood sampling was performed over 3 days following primaquine and 36 days following dihydroartemisinin-piperaquine dosing. Pharmacokinetic assessment was done with a noncompartmental approach. The drugs were well tolerated. There were no statistically significant differences in dihydroartemisinin-piperaquine pharmacokinetics with or without primaquine. Dihydroartemisinin-piperaquine coadministration significantly increased plasma primaquine levels; geometric mean ratios (90% confidence interval [CI]) of primaquine combined versus primaquine alone for maximum concentration (Cmax), area under the concentration-time curve from 0 h to the end of the study (AUC0–last), and area under the concentration-time curve from 0 h to infinity (AUC0–∞) were 148% (117 to 187%), 129% (103 to 163%), and 128% (102 to 161%), respectively. This interaction is similar to that described recently with chloroquine and may result in an enhanced radical curative effect. (This study has been registered at ClinicalTrials.gov under registration no. NCT01525511.)

MATERIALS AND METHODS

Subjects. Sixteen healthy Thai adults (11 female, 5 male) between 18 and 60 years of age were recruited. They were nonsmokers and were judged healthy based on clinical history, physical examination, and baseline screening results in hematology, biochemistry, urinalysis, and electrocardiogram (ECG), with a corrected QT (QTc) (Fridericia) interval of <450 ms. Exclusion criteria included a history of drug allergy, alcohol or substance abuse, concomitant medication intake, G6PD deficiency as detected by Beutler’s dye test, or positive HIV, hepatitis B, or hepatitis C serology. Female subjects were of nonchildbearing potential or, if of childbearing potential, had a negative serum pregnancy test and agreed to use effective contraceptive methods during the study. The study protocol was approved by the ethics committee of the Faculty of Tropical Medicine, Mahidol University (reference number TMEC 12–004, approval number...
Dihydroartemisinin-piperaquine, and vice versa in the third admission. Participants received a single dose of 2 tablets of primaquine together with 3 tablets of testing program with satisfactory performance (http://www.wwarn.org/). The laboratory participates in the WorldWide Antimalarial Resistance Network (WWARN) quality control and assurance proficiency assessments. The laboratory is accredited by the Department of Clinical Pharmacology, Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand in the first admission. The volunteers were randomized into the pharmacokinetic unit at the Hospital for Tropical Diseases the evening before the study began. Subjects were given a light standard meal (~200 kcal with 8 g fat) 30 min before each drug dose and were allowed to eat 4 h after administration of the study drug. Water and/or soft drinks without caffeine were permitted 2 h postdose. Study drugs were taken orally with a glass of water. Vital signs were checked every 4 h after dosing. Both groups received 2 tablets of primaquine phosphate (15 mg base/tablet; Government Pharmaceutical Organization, Thailand) in the first admission. In the second admission, one group (n = 8) was given a single dose of 3 tablets of dihydroartemisinin-piperaquine (Eurartesim; Sigma-Tau Industrie Farmaceutiche Riunite S.p.A.) only (40 mg dihydroartemisinin/320 mg piperaquine phosphate per tablet), and the other group (n = 8) received a single dose of 2 tablets of primaquine together with 3 tablets of dihydroartemisinin-piperaquine, and vice versa in the third admission. The washout periods between doses were >1 week after primaquine and >8 weeks after dihydroartemisinin-piperaquine–containing treatments. Electrocardiograms were recorded at 0, 1, 2, 4, 8, 12, and 24 h postdose in each admission. Methemoglobin was measured at each pharmacokinetic blood sampling time (see below) using a noninvasive monitoring machine (Masimo pulse oximeter, SpMet).

Pharmacokinetic sampling. For the pharmacokinetic assessment of all drugs, blood samples (2 ml) were collected into fluoride-oxalate tubes at 0 (predose), 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 h and on day 3 (48 h to 54 h). An indwelling catheter was used for the multiple serial blood collections from 0 to 12 h postdose. Additional blood samples were taken for piperaquine measurements on days 4, 7, 11, 15, 22, and 36. After collection, blood samples were centrifuged for 7 min at 2,000 × g at 4°C, and plasma was stored at −70°C or lower. All samples were transferred to the Department of Clinical Pharmacology,Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, for plasma drug measurements. The laboratory participates in the WorldWide Antimalarial Resistance Network (WWARN) quality control and assurance proficiency testing program with satisfactory performance (http://www.wwarn.org/toolkit/qaqc).

Drug analysis. Dihydroartemisinin and piperaquine plasma concentrations were quantified using high-performance liquid chromatography linked with tandem mass spectrometry according to previously published methods (11, 12). The limit of quantification was 2.0 ng/ml for dihydroartemisinin and 1.50 ng/ml for piperaquine, respectively. Primaquine and carboxyprimaquine plasma concentrations were quantified using solid-phase extraction and high-performance liquid chromatography with mass spectrometry detection (reference 13 and; unpublished data). The limits of quantification were 1.14 ng/ml and 4.88 ng/ml for primaquine and carboxyprimaquine, respectively. Three replicates of quality control samples at low, middle, and high concentrations were analyzed within each batch of clinical samples to ensure precision and accuracy during drug measurements. The total precision (i.e., relative standard deviation [SD]) for all drug measurements was <9.0% during drug quantification.

Pharmacokinetic analysis. Individual subject concentration-time data were evaluated using a noncompartmental analysis approach as implemented in WinNonlin version 6.3 (Pharsight Corporation, USA). The terminal elimination rate constant (λ2) was estimated by log-linear best-fit regression of the observed plasma concentrations in the terminal elimination phase, without data point removal. Visual inspection of all concentration-time profiles were performed to ensure an adequate fit to the observed data. Total exposure up to the last measured concentration (AUC0–last) was calculated using the linear trapezoidal method for ascending concentrations and the logarithmic trapezoidal method for descending concentrations. Exposure was extrapolated from the last observed concentration to infinity by Clast/λ2 for each subject to compute total drug exposure (AUC0–∞). The terminal elimination half-life (t1/2) was estimated by ln 2/λ2. The maximum plasma drug concentration (Cmax) and time to maximum concentration (Tmax) were taken directly from the observed data. The total apparent volume of distribution (V/F) and oral clearance (CL/F) were computed individually according to the equations V/F = dose/(λ2 × AUC) and CL/F = dose/AUC, respectively.

Pharmacokinetic parameter estimates were compared between a single dose of each study drug administered alone and in combination using the Wilcoxon signed-rank test in STATA v.11. An analysis of variance (ANOVA) was carried out on the log-transformed pharmacokinetic exposure parameters Cmax, AUC0–last, and AUC0–∞ to assess the bioequivalence of the drug administered alone versus that in combination. The effects of coadministration, the sequence of administrations, and subjects were examined in an adjusted model. The point estimate of the geometric mean ratio and the residual variability from the ANOVA were used to calculate the 90% confidence intervals (CIs) around the mean. The U.S. FDA criteria for assuming no interaction when the drugs are coadministered were met if the confidence intervals (90% CI) for the geometric mean ratios were retained within 80% to 125% (14).

Safety analysis. Safety was analyzed based on adverse events (AEs), physical examination, vital signs, clinical laboratory parameters, 12-lead electrocardiogram (ECG) findings, and methemoglobin levels. A >30-ms change from baseline in QTc interval (using Fridericia’s correction) was specified prospectively as clinically significant, and any subject with this change at any time point was noted.

The safety and tolerability of primaquine and dihydroartemisinin-piperaquine were assessed by using the Wilcoxon matched-pair signed-rank test for continuous variables or McNemar’s exact test for categorical variables when drugs were given alone or in combination. The frequencies (%) of adverse events and serious adverse events, with particular attention to those of potential clinical concern, were presented by treatment group and reported by visit so that any effect of the addition of primaquine and...
reexposure to piperquine could be assessed. All liver function test (LFT) parameters were also compared within each visit by treatment (to assess the addition of primaquine) using the Mann-Whitney U test and within groups (to assess reexposure to dihydroartemisinin-piperquine) using the Wilcoxon matched-pair signed-rank test. Subjects were analyzed as groups (to assess reexposure to dihydroartemisinin-piperquine alone [Kendall’s tau = 0.0017]) and their changes from before dosing at each time point up to 24 h are shown in Table 2. There was a small (median, 2%) but significant shortening of the QTc (Fridericia) interval following dihydroartemisinin-piperquine treatment with (8 ms) or without (7 ms) primaquine concomitantly administered, which was maximal at 4 h after dosing compared to primaquine alone (P = 0.0009 and P = 0.0027, respectively). This correlated with the primaquine Cmax (correlation coefficient for maximum QTc prolongation following dihydroartemisinin-piperquine administration [Kendall’s tau] = 0.48, P = 0.01; in combination with primaquine, P = 0.0649). The addition of primaquine to dihydroartemisinin-piperquine did not affect the magnitude of QTc prolongation (P = 0.5695) (Table 3). Two female subjects (38 and 31 years old) had a QTc interval marginally above 450 ms (450.3 and 450.51 ms, respectively) at 4 h after dihydroartemisinin-piperquine administration. QTc interval prolongations from a predose baseline of >30 ms (32 and 33 ms, respectively) were observed in 2 subjects 4 h after dihydroartemisinin-piperquine administration. All subjects had methemoglobin levels of <3% at all times during the study. Three severe adverse events (SAEs) were reported by 3 subjects. All were deemed unrelated to the study drug or study procedure. One subject had a rickettsial infection, 1 subject had unstable angina with dizziness with nonspecific ECG changes, and the third subject had acute bronchitis. All of the SAEs required hospitalization, and all resolved subsequently. Six other minor AEs were reported by 5 subjects and were considered unrelated to the study drug. All AEs resolved subsequently.

**Pharmacokinetic analysis.** There were no statistically significant differences in dihydroartemisin and piperquine pharmacokinetics when administered with or without primaquine (Fig 1; Table 4). The geometric mean ratios and 90% CIs of dihydroartemisin and piperaquine administered with and without primaquine for the logarithmically transformed AUC0–last and AUC0–∞ values were within the limits accepted for bioequivalence (Table 5; Fig. 2). However, the variability in Cmax values was too great to assume bioequivalence.

There were significant changes in the pharmacokinetics of primaquine and its major metabolite carboxypiperaquine when administered with dihydroartemisinin-piperquine (Fig 1; Table 6). Combined administration with dihydroartemisinin-piperquine resulted in significantly lower primaquine CL/F (P = 0.0229) and V/F (P = 0.0013) values than administration alone, leading to significantly higher Cmax (P = 0.0019) and AUC0–last (P = 0.0200) values. This also resulted in a shorter primaquine t1/2 (P = 0.0005) than with administration alone. Geometric mean ratios (90% CI) of primaquine administered with and without dihydroartemisinin-piperquine for Cmax, AUC0–last, and AUC0–∞ were 148%

**TABLE 2** QTc intervals (Fridericia’s correction) at predose of each regimen and changes from before dosing to 24 h afterward

| Dosing time | QTc change (ms) for treatment with a | P value b for: | Combination |
|-------------|-------------------------------------|----------------|-------------|
|             | Primaquine alone | DHA-PQP alone | Primaquine versus DHA-PQP | Primaquine versus combination | DHA-PQP versus combination |
| Predose     | 417.9 (17.9)  | 420.4 (13.7) | 0.264 | 0.265 | 0.063 |
| 1 h         | −7.91 (−20.8 to −2.00) | −1.66 (−10.0 to 0.41) | −3.55 (−9.56 to 1.24) | 0.088 | 0.017 | 0.959 |
| 2 h         | −10.4 (−20.7 to −0.59) | −1.58 (−11.5 to 4.40) | 0.06 (−7.22 to 5.54) | 0.163 | 0.007 | 0.326 |
| 4 h         | −3.37 (−7.98 to 4.97) | 7.08 (2.16 to 22.1) | 8.28 (2.76 to 14.6) | 0.002 | 0.004 | 0.918 |
| 8 h         | −14.2 (−19.9 to −7.84) | −0.001 (−10.8 to 3.36) | 3.79 (−8.74 to 9.00) | 0.023 | 0.020 | 0.408 |
| 12 h        | −8.85 (−17.6 to −2.45) | −0.52 (−10.3 to 6.76) | −0.31 (−8.20 to 5.26) | 0.034 | 0.070 | 0.717 |
| 24 h        | −8.09 (−13.3 to −2.85) | 2.99 (−9.93 to 9.95) | −0.95 (−2.42 to 12.0) | 0.030 | 0.004 | 0.234 |

a n = 16 per treatment group. Values are shown as median (interquartile range) or mean (SD). DHA-PQP, dihydroartemisinin-piperquine; combination, primaquine plus dihydroartemisinin-piperquine.

b Compared using paired t test for predose and Wilcoxon matched-paired signed-rank test for all others.

**TABLE 3** The maximum electrocardiograph QTc (Fridericia’s correction) readings within 24 h after drug administration, time of onset and changes from baseline

| QTc reading | Treatment | P value |
|-------------|-----------|---------|
| Time to onset (h) | Primaquine alone | DHA-PQP alone | Combination | Primaquine versus DHA-PQP | Primaquine versus combination | DHA-PQP versus combination |
| 4 (1–24) | 4 (2–24) | 4 (2–24) | 0.0387 | 0.1474 | 0.7266 |
| 0.53 (−3.85 to 2.07) | 2.10 (−1.85 to 8.13) | 2.73 (−0.22 to 8.30) | 0.0027 | 0.0009 | 0.5695 |

a Values are shown as median (range). n = 16 per treatment group. DHA-PQP, dihydroartemisinin-piperquine; combination, primaquine plus dihydroartemisinin-piperquine.
Similarly, when primaquine was administered in combination with dihydroartemisinin-piperaquine, there were also significantly higher carboxyprimaquine exposures ($C_{\text{max}}$, $P = 0.0032$; $\text{AUC}_{0-\text{last}}$, $P = 0.0262$) and lower $V/F$ ($P = 0.0019$) and shorter $t_{1/2}$ ($P = 0.0084$) values than with administration alone. The geometric mean ratios (90% CI) of carboxyprimaquine administered with and without dihydroartemisinin-piperaquine for $C_{\text{max}}$, $\text{AUC}_{0-\text{last}}$, and $\text{AUC}_{0-\infty}$ were 133% (106 to 168%), 126% (99.3 to 160%), and 119% (92.8 to 153%), respectively (Fig. 2). This follows the pattern of alteration in primaquine pharmacokinetics, confirming a significant drug-drug interaction between primaquine and dihydroartemisinin-piperaquine.

**DISCUSSION**

The values of the pharmacokinetic parameters estimated for dihydroartemisinin and piperaquine in this study are mostly comparable to those of a previous study by Chinh and coworkers (10) (geometric means of dihydroartemisinin: $T_{\text{max}}$, 1.5 h; $t_{1/2}$, 1.01 h; $CL/F$, 2.21 liters/h; $V/F$, 5.53 liters/kg; $C_{\text{max}}$, 364 ng/ml; $\text{AUC}_{0-\text{last}}$, 812 ng · h/ml; $\text{AUC}_{0-\infty}$, 817 ng · h/ml). Table 4 shows the pharmacokinetic parameters of dihydroartemisinin and piperaquine administered alone and in combination with primaquine.

![FIG 1 Mean venous plasma concentration-time curves of primaquine (A), carboxyprimaquine (B), dihydroartemisinin (C), and piperaquine (D) in healthy volunteers. Error bars indicate SDs.](http://aac.asm.org/)

**TABLE 4** Pharmacokinetic parameters of dihydroartemisinin and piperaquine administered alone and in combination with primaquine

| Parameter | Dihydroartemisinin | Piperaquine |
|-----------|-------------------|-------------|
|           | Alone | Combination | $P$ value | Alone | Combination | $P$ value |
| Total dose (mg/kg) | 1.87 (1.68–2.22) | 1.87 (1.68–2.22) | NA | 8.65 (7.76–10.3) | 8.65 (7.76–10.3) | NA |
| $C_{\text{max}}$ (ng/ml) | 364 (184–792) | 348 (194–961) | 0.3011 | 491 (129–1,270) | 397 (127–1,200) | 1.0000 |
| $T_{\text{max}}$ (h) | 1.50 (1.00–2.00) | 1.50 (0.50–3.00) | 1.0000 | 4.00 (3.00–4.00) | 4.00 (3.00–6.00) | 0.7419 |
| $CL/F$ (liters/h/kg) | 2.21 (0.96–5.01) | 2.23 (0.87–5.52) | 0.6051 | 0.450 (0.17–0.73) | 0.441 (0.275–0.554) | 0.1477 |
| $V/F$ (liters/kg) | 5.53 (2.67–11.3) | 5.89 (2.70–11.0) | 0.1788 | 225 (120–593) | 265 (139–339) | 1.0000 |
| $t_{1/2}$ (h) | 1.97 (1.13–2.67) | 1.81 (1.13–2.84) | 0.1788 | 390 (224–669) | 449 (206–610) | 0.6417 |
| $\text{AUC}_{0-\text{last}}$ (ng · h/ml) | 812 (394–2,010) | 890 (338–2,210) | 1.0000 | 17,400 (8,120–36,800) | 15,400 (12,200–31,200) | 0.7960 |
| $\text{AUC}_{0-\infty}$ (ng · h/ml) | 817 (398–2,030) | 899 (361–2,250) | 0.9176 | 20,400 (11,400–57,300) | 19,800 (15,400–35,900) | 0.6417 |
| Ext. AUC (%) | 1.45 (0.253–4.19) | 1.12 (0.338–2.81) | 0.0703 | 17.9 (6.72–35.7) | 20.8 (4.95–35.2) | 0.6051 |

*a C$_{\text{max}}$ maximum observed plasma concentration after oral administration; $T_{\text{max}}$ observed time to reach $C_{\text{max}}$; $CL$, elimination clearance; $V$, apparent volume of distribution; $t_{1/2}$, terminal elimination half-life; $\text{AUC}_{0-\text{last}}$, total exposure up to the last measured concentration; $\text{AUC}_{0-\infty}$, predicted area under the plasma concentration-time curve after the last dose from zero time to infinity; Ext. AUC, percentage of $\text{AUC}_{0-\infty}$ extrapolated from the last observation to infinity.

*b Data are presented as median (range). $n = 16$ per treatment group. NA, not available.
The present study showed lower clearance and volume of distribution of dihydroartemisinin and consequently higher AUC and $C_{\text{max}}$ values (geometric means: AUC $0–\text{last}$, 817 versus 370 ng · h/ml; $C_{\text{max}}$, 364 versus 159 ng/ml). The present study also showed a higher $C_{\text{max}}$ but a similar AUC of piperaquine ($C_{\text{max}}$, 491 versus 204 ng/ml; AUC $0–\text{last}$, 20,400 versus 19,929 ng · h/ml). These differences observed between studies may reflect differences in the volunteers’ age, diet, or gender and/or the play of chance given the large interindividual variability and small sample sizes. Piperaquine absorption may be enhanced when administered with a high-fat meal (15,16), although small amounts of fat have little effect on piperaquine bioavailability (17,18). In this study, no drug-drug interactions were observed in dihydroartemisinin and piperaquine pharmacokinetics as a result of primaquine coadministration. The AUCs of dihydroartemisinin and piperaquine were all within the 90% CI of the geometric means ratio of 80 to 125%.

When administered alone, primaquine pharmacokinetic results were comparable to those of the previous studies. Elmes et al. (19) reported mean (SD) plasma primaquine values in healthy Australian men and women, respectively, as follows: $C_{\text{max}}$ of 93 (26) and 115 (38) ng/ml, AUC $0–\text{last}$ of 1,105 (475) and 1,240 (444) ng · h/ml, and CL/F of 0.34 (0.12) and 0.39 (0.14) liters/h/kg. Binh et al. (20) reported a median plasma primaquine $C_{\text{max}}$ of 122 ng/ml, $T_{\text{max}}$ of 2.0 h, and $t_{1/2}$ of 6.1 h in healthy Vietnamese volunteers. Coadministration of primaquine with dihydroartemisinin-piperaquine resulted in significantly higher exposure, higher $C_{\text{max}}$, lower $V/F$, and shorter $t_{1/2}$ of both primaquine and carboxyprimaquine.

This pharmacokinetic interaction is similar in direction to that recently observed with other antimalarials. Coadministration of primaquine with chloroquine and with pyronaridine-artesunate demonstrated similar changes in primaquine pharmacokinetics (13; unpublished observations). In studies conducted >60 years ago, mecaprine (quinacrine [Atabrine]), an acridine with structural similarities to chloroquine, markedly elevated levels of pamaquine (plasmoquine, an 8-aminoquinoline predecessor of primaquine) (21,22). Thus, several structurally related antimalarials, all with extensive tissue distribution and very slow elimination, elevate plasma concentrations of the 8-aminoquinoline drugs. Tissue displacement is therefore one potential mechanism to explain the interaction, and the likely interacting drug is therefore piperaquine rather than dihydroartemisinin. Whether this involves competition for transporters, such as that demonstrated in a study on the effect of rifampin, an organic anion-transporting polypeptide (OATP) inhibitor, on digoxin metabolism in rats

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### TABLE 5 Bioequivalence analysis of dihydroartemisinin, piperaquine, primaquine and carboxyprimaquine after administration of dihydroartemisinin-piperaquine and primaquine alone and in combination

| Parameter
table_cell | Dihydroartemisinin | Piperaquine | Primaquine | Carboxyprimaquine |
|--------------|---------------------|-------------|------------|------------------|
| $C_{\text{max}}$ (ng/ml) | 111 (92.1–134) | 98.1 (74.6–129) | 148 (117–187) | 133 (106–168) |
| AUC $0–\text{last}$ (ng · h/ml) | 100 (86.7–116) | 105 (90.3–121) | 129 (103–163) | 126 (99.3–160) |
| AUC $0–\text{last}$ (ng · h/ml) | 99.9 (86.5–115) | 105 (91.4–121) | 128 (102–161) | 119 (92.8–153) |

$C_{\text{max}}$, maximum observed plasma concentration; AUC $0–\text{last}$, total exposure up to the last measured concentration; AUC $0–\text{last}$, predicted area under the plasma concentration time curve after the last dose from zero time to infinity.

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**FIG 2** Forest plots of the geometric mean ratios (90% CI) of the drug administered with and without interacting drug for the logarithmically transformed $C_{\text{max}}$, AUC $0–\text{last}$ and AUC $0–\text{last}$. Vertical dashed lines represent the U.S. FDA criteria of 80 to 125% for assuming bioequivalence.
could explain the discrepancy between CL/F and V/F. This scenario is based on the assumption that primaquine is passively absorbed from the gut but actively transported into hepatocytes. An increase in the bioavailability of primaquine seems less likely given that volunteer studies suggest near-100% oral bioavailability for primaquine (24). Primaquine metabolism involves monoamine oxidase A (25) and cytochrome P450 (CYP) isozymes, especially 2C19 (25), 2D6, and 3A4 (25, 26). Piperquine inhibits CYP3A4 (27, 28) and CYP2C19 (27), and metabolic inhibition cannot be excluded as a contributor to reduced primaquine clearance. As the active metabolites of primaquine are produced via CYP2D6, a different route to the monoamine oxidase pathway which produces carboxyprimaquine, the relevance of these findings to primaquine’s pharmacodynamic effects remains to be determined. However, by inference, the efficacy synergy for radical clearance (30).

In conclusion, coadministration of dihydroartemisinin-piperaquine and primaquine was well tolerated in healthy adult subjects. This combination did not result in any significant pharmacokinetic alterations of dihydroartemisinin and piperquine but increased plasma concentrations of primaquine. Further study is required to determine how this affects primaquine pharmacodynamics, but there seems to be no reason to not recommend this combination.

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MMV reviewed and commented on the study protocol but had no part in its implementation, the analysis or interpretation of the study, or the decision to publish the results.

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