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Differential Cytochrome P450 2D Metabolism Alters Tafenoquine Pharmacokinetics

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Cytochrome P450 (CYP) 2D metabolism is required for the liver-stage antimalarial efficacy of the 8-aminoquinoline molecule tafenoquine in mice. This could be problematic for *Plasmodium vivax* radical cure, as the human CYP 2D ortholog (2D6) is highly polymorphic. Diminished CYP 2D6 enzyme activity, as in the poor-metabolizer phenotype, could compromise radical curative efficacy in humans. Despite the importance of CYP 2D metabolism for tafenoquine liver-stage efficacy, the exact role that CYP 2D metabolism plays in the metabolism and pharmacokinetics of tafenoquine and other 8-aminoquinoline molecules has not been extensively studied. In this study, a series of tafenoquine pharmacokinetic experiments were conducted in mice with different CYP 2D metabolism statuses, including wild-type (WT) (reflecting extensive metabolizers for CYP 2D6 substrates) and CYP_{mouse} 2D knockout (KO) (reflecting poor metabolizers for CYP 2D6 substrates) mice. Plasma and liver pharmacokinetic profiles from a single 20-mg/kg of body weight dose of tafenoquine differed between the strains; however, the differences were less striking than previous results obtained for primaquine in the same model. Additionally, the presence of a 5,6-*ortho*-quinone tafenoquine metabolite was examined in both mouse strains. The 5,6-*ortho*-quinone species of tafenoquine was observed, and concentrations of the metabolite were highest in the WT extensive-metabolizer phenotype. Altogether, this study indicates that CYP 2D metabolism in mice affects tafenoquine pharmacokinetics and could have implications for human tafenoquine pharmacokinetics in polymorphic CYP 2D6 human populations.

The 8-aminoquinoline class of antimalarial compounds is the only molecular scaffold with proven efficacy against relapsing strains of malaria (1–5). Currently, the only FDA-approved drug from this class that is available for clinical use is primaquine. Primaquine has been utilized for over 6 decades in treating malaria (6). Despite the long history of primaquine therapy for malaria treatment, primaquine has several disadvantages, including the hemolytic toxicity associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency; its relatively short elimination half-life in humans, which requires daily administration; and the potential requirement for cytochrome P450 (CYP) 2D6-mediated activation for radical curative activity (7–11). The 8-aminoquinoline molecule tafenoquine, currently under late-stage clinical development, has a significantly longer elimination half-life than primaquine (12–16) and has single-dose radical curative activity in humans (3). Tafenoquine is also being developed as a chemoprophylactic agent and has demonstrated efficacy against *Plasmodium vivax* and *Plasmodium falciparum* (17–21). Despite the pharmacological advantages of tafenoquine over primaquine, both molecules seem to have the same pharmacogenomic liability of CYP 2D6-mediated activation for liver-stage antimalarial activity (7, 10, 22, 23) and are not free of hemolytic liability in G6PD deficiency (24). This recent discovery suggests that the 8-aminoquinoline class of molecules require CYP 2D6 metabolism for efficacy. This CYP 2D6 requirement has unknown pharmacokinetic (PK) and pharmacodynamic implications in humans for the 8-aminoquinolines. Recently, the studies of Potter et al. and Bennett et al. demonstrated clear differences in primaquine pharmacokinetic profiles in both mouse and human models of differential CYP 2D6 metabolism (7, 25). Diminished CYP 2D6-mediated clearance resulted in higher concentrations of the parent primaquine *in vivo*. Prior to this study, there were no reports of the pharmacokinetic profiles of tafenoquine in animals or humans with decreased CYP 2D6 metabolism.

We sought to extend our previous studies of CYP 2D6 (22) involvement in tafenoquine activation by determining the pharmacokinetic consequences of altered CYP 2D6 metabolism. To explore tafenoquine pharmacokinetics in the context of differential CYP 2D6 metabolism, a series of pharmacokinetic experiments were conducted in mice with different CYP 2D6 substrate metabolizer statuses. The two strains of mice selected were wild-type (WT) C57BL/6 mice, and CYP 2D knockout (KO) C57BL/6 mice (for a description of the KO strain, see reference 26). WT

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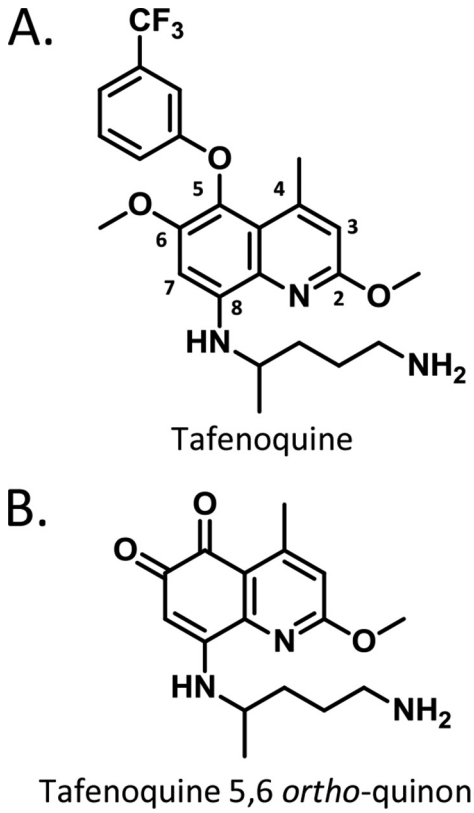

FIG 1 Compounds utilized in the tafenoquine pharmacokinetic study. Shown are the structures of tafenoquine (A) and 5,6-*ortho*-quinone (B) of the tafenoquine standards utilized. The quinoline ring of tafenoquine is numbered for reference.

mice express CYP 2D22, which is the mouse cytochrome P450 ortholog closest to human CYP 2D6 (27). Tafenoquine is active when tested in a liver-stage malaria model against *Plasmodium berghei* that utilizes the WT C57BL/6 mouse strain (10, 22). The CYP 2D KO strain contains a deletion of the entire CYP 2D gene cassette and therefore cannot express CYP 2D22. Tafenoquine is inactive against liver stages of *P. berghei* when tested in KO mice (22). These two strains replicate the human extensive-metabolizer (WT mice) and poor-metabolizer (KO mice) CYP 2D6 phenotypes.

Pharmacokinetic measurements of tafenoquine were made, utilizing the analytical standards illustrated in Fig. 1. The structure of the parent tafenoquine is shown in Fig. 1A. In addition to monitoring for the parent tafenoquine, the presence of a 5,6-*ortho*-quinone species was also investigated. The structure of the tafenoquine 5,6-*ortho*-quinone is shown in Fig. 1B. Tafenoquine has a bulky aryl substitution at the 5 position of the quinoline ring, while primaquine has a hydrogen. It has been proposed that hydroxylation of primaquine at the 5 position produces a reactive metabolite(s) that is likely responsible for efficacy and toxicity (10, 28–31). The slow kinetics of metabolic dearylation of the carbon at the 5 position of the quinoline core (C5) of tafenoquine is challenging to study using traditional *in vitro* metabolism techniques due to the extreme stability of the molecule in microsomal preparations *in vitro*. The metabolite shown in Fig. 1B for tafenoquine corresponds to the stable oxidation product of the 5-dearylation pathway. A related metabolic pathway (C5 hydroxylation) was recently shown to be affected by mouse CYP 2D metabolism for primaquine (25). The existence of such a phenolic metabolite for tafenoquine has not been demonstrated *in vivo*. The existence of this C5 phenolic metabolite for tafenoquine, along with other phenolic metabolites similar to those produced by primaquine metabolism, would further indicate a common CYP 2D6-mediated metabolic pathway for 8-aminoquinoline radical curative activity.

MATERIALS AND METHODS

Materials and reagents. Tafenoquine succinate and the 5,6-*ortho*-quinone metabolite standards were obtained from the Walter Reed Army Institute of Research chemical repository.

Pharmacokinetic measurements. Experiments were conducted as described by Potter et al. (25). Briefly, male 6- to 14-week-old C57BL/6 and 2D knockout C57BL/6 mice (Taconic, Hudson, NY) were used for PK evaluations. On arrival, the animals were acclimated for 7 days in quarantine. The animals were housed in a cage maintained at a temperature range of 64 to 79°F and 34 to 68% relative humidity with a 12-h light/dark cycle. Food and water were provided *ad libitum* during quarantine and throughout the study. The animals were fed a standard rodent maintenance diet. All animal studies were performed under IACUC-approved protocols. These protocols detail the experimental procedures and designs, as well as the number of animals used. The research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the National Research Council publication *Guide for the Care and Use of Laboratory Animals*, 2011 edition.

PK studies were performed using oral administration. At each time point (0, 0.5, 2, 4, 8, 24, 48, 96, 168, 240, and 336 h), three male mice from each group were dosed with tafenoquine (20 mg/kg of body weight based on free base molecular weight). Tafenoquine succinate was reconstituted in double-distilled H_2O (ddH_2O) and administered at 100 μl/20 g. For each time point, whole-blood and liver samples were collected. Plasma was obtained from whole blood (500 μl) collected by cardiac puncture; 500 μl of 1,000-USP unit/ml heparin (Hospira, Lake Forest, IL) was added to the whole-blood samples to prevent coagulation prior to plasma isolation via centrifugation. The isolated plasma samples were stored at −80°C until liquid chromatography-tandem mass spectrometry (LC–MS-MS) analysis. Liver samples were extracted for each time point and immediately preserved on dry ice and stored at −80°C until homogenization, extraction, and LC–MS-MS analysis.

LC–MS-MS analysis. All samples (5 μl each) were analyzed using an AB Sciex 4000 QTrap mass spectrometer (AB Sciex, Framingham, MA, USA). Chromatographic separations were achieved using a Waters XTerra MS C_{18} (50-mm by 2.1-mm; 3-μm) analytical column, a Waters I class liquid chromatography system flowing at 0.40 ml/min, and a 4-min linear gradient from 5 to 98% acetonitrile (0.1% formic acid). The analytes were detected with electrospray ionization in the positive ion mode. The mass spectrometry conditions were optimized for each analyte. The peak areas were extracted using AB Sciex Analyst software. Standard curves (0.5 to 1,000 ng/ml) and quality control samples were prepared by spiking blank mouse plasma or liver homogenate and serially diluting it to the desired concentration. Liver samples were diluted with 5 volumes of water (1 ml/g of liver) and homogenized prior to extraction. Standard curves, quality control samples, and PK samples were prepared for analysis by extraction with 2 volumes of acetonitrile with an internal standard (mefloquine) for each volume of sample. Each sample was vortexed and centrifuged at 16,000 × *g* for 10 min at 4°C. The supernatant was transferred to a 96-well plate for LC–MS-MS analysis.

Pharmacokinetic parameter determination. Pharmacokinetic parameter determination was performed as described by Potter et al. (25). Briefly, pharmacokinetic parameters for tafenoquine and the 5,6-*ortho*-quinone in plasma and liver were determined using noncompartmental analysis via the Phoenix-WinNonlin software package (version 6.3; Phar-

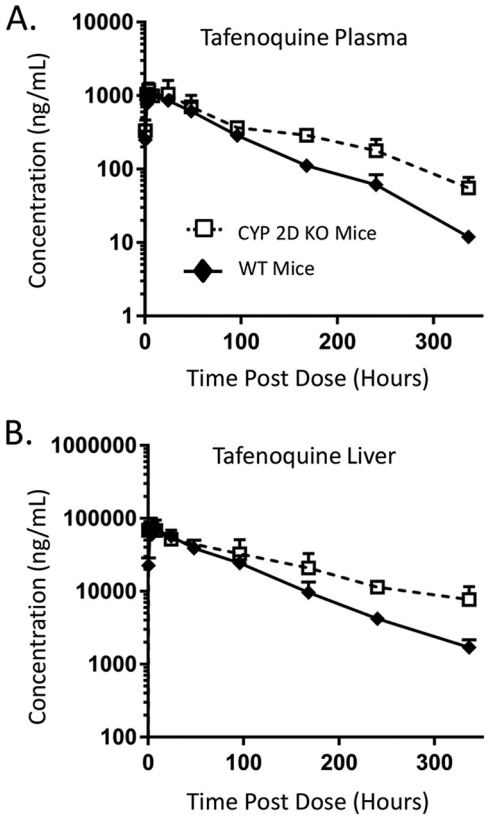

FIG 2 Tafenoquine pharmacokinetics in WT and KO mice. (A) Plasma pharmacokinetic profiles for tafenoquine in CYP 2D KO and WT mice. (B) Liver pharmacokinetic profiles for tafenoquine in CYP 2D KO and WT mice. The error bars show the standard deviations from replicate analyses (WT, $n = 3$; KO, $n = 4$).

sight Corp., Mountain View, CA). The maximum concentration of drug in plasma (C_{max}) and time to maximum concentration (T_{max}) were obtained directly from the plasma and liver drug concentration-time curves. The elimination half-life ($t_{1/2}$) was calculated from $\ln_2/k_{el}$, which is the elimination rate constant calculated from the concentration-time plot. The area under the curve (AUC) was determined by the linear trapezoidal rule, with extrapolation to infinity (AUC_{inf}) based on the concentration of the last time point divided by the terminal rate constant. The apparent clearance rate (CL) was determined by dividing the dose by the AUC_{inf}. The mean residence time (MRT) was determined by dividing the area under the first moment of the curve (AUMC) by the AUC. The relative total systemic clearance (CL/*F*) and apparent volume of distribution during steady state (V_Z/F) were also estimated from the noncompartmental analysis.

RESULTS

Plasma pharmacokinetics of tafenoquine in WT and KO mice. The pharmacokinetic profile of tafenoquine was assessed in the WT and KO mouse strains after a single oral 20-mg/kg dose of tafenoquine. Figure 2A shows plasma tafenoquine pharmacokinetics in the WT strain and in the KO strain. In both cases, tafenoquine remained in the plasma throughout the duration of the sampling period (336 h). However, while tafenoquine concentrations in the KO strain were initially similar to those observed in the WT strain, at the later time points (>100 h), the KO strain had higher plasma tafenoquine concentrations than the WT strain.

The pharmacokinetic parameters from the profiles shown in Fig. 2A were determined and are indicated in Table 1. The plasma half-life of tafenoquine in the KO strain was longer than in the WT strain (72.4 h for the KO versus 53.8 h for the WT). However, it was not statistically significantly different ($P = 0.1$). There were no notable differences in the T_{max} ($P = 0.4$) or C_{max} ($P = 0.3$) values between the two strains for plasma. The calculated AUC_{0-inf} values (123.2 h · μg/ml for the KO versus 85.6 h · μg/ml for the WT; $P = 0.03$) were higher in plasma for tafenoquine in the KO strain than in the WT mice. Apparent clearance was decreased in the KO strain compared to the WT animals for plasma profiles (164.5 ml/h/kg for the KO versus 238.0 ml/h/kg for the WT; $P = 0.01$). The plasma pharmacokinetic results for tafenoquine in the two strains indicate that decreased CYP 2D metabolism in the KO mice had an effect on the terminal elimination kinetics of tafenoquine, resulting in higher overall exposure of the unmodified parent molecule.

Liver pharmacokinetics of tafenoquine in WT and KO mice. Tafenoquine radically curative antimalarial activity is presumed to occur in the host liver against the hypnozoite stage of the *Plasmodium* parasite. To further investigate tafenoquine pharmacokinetics in the presumed target tissue and in the context of differential CYP 2D metabolism, liver pharmacokinetic measurements were made for tafenoquine. The liver results are shown in Fig. 2B. Despite higher overall concentrations of tafenoquine in liver versus plasma, the overall profiles of tafenoquine in the KO and WT strains were similar to those observed from plasma measurements. Initially, tafenoquine levels were comparable in both strains; however, over several weeks, the concentration of tafenoquine fell more rapidly in the WT mice than in the KO strain.

TABLE 1 Pharmacokinetic parameters of tafenoquine tested in mice[a]

Parameter	Value		
	WT ($n = 3$)	CYP 2D KO ($n = 4$)	*P* value (WT vs KO)
Plasma			
$t_{1/2}$ (h)	53.8 ± 3.5	72.4 ± 15.5	0.1
T_{max} (h)	5.0 ± 3.0	10.0 ± 9.8	0.4
C_{max} (μg/ml)	1.2 ± 0.02	1.4 ± 0.3	0.3
AUC_{0-last} (h · μg/ml)	84.7 ± 14.1	113.9 ± 6.3	0.01
AUC_{0-inf} (h · μg/ml)	85.6 ± 14.1	123.2 ± 17.4	0.03
V_Z/F (liter/kg)	18.5 ± 3.7	19.9 ± 1.6	0.5
CL/*F* (ml/h/kg)	238.0 ± 35.9	164.5 ± 20.8	0.01
MRT_{0-last} (h)	70.9 ± 0.4	94.1 ± 4.9	0.0005
Liver			
$t_{1/2}$ (h)	83.5 ± 2.3	80.1 ± 12.9	0.8
T_{max} (h)	4.7 ± 3.1	4.5 ± 2.5	0.8
C_{max} (μg/ml)	29.3 ± 0.6	85.8 ± 15.5	0.002
AUC_{0-last} (h · μg/ml)	3,242.2 ± 602.4	9,328.6 ± 2,557.3	0.01
AUC_{0-inf} (h · μg/ml)	3,447.9 ± 659.6	10,317.7 ± 2,009.5	0.002
V_Z/F (liter/kg)	0.7 ± 0.1	0.2 ± 0.08	0.002
CL/*F* (ml/h/kg)	6.0 ± 1.2	2.0 ± 0.4	0.002
MRT_{0-last} (h)	96.2 ± 6.0	88.5 ± 12.3	0.4

[a] Pharmacokinetic parameters are shown for the mouse strains from plasma and liver determinations. The units for each parameter are indicated, and the errors shown are the standard deviations from triplicate analyses. *P* values are also indicated from comparison of the KO versus the WT C57BL/6 mice.

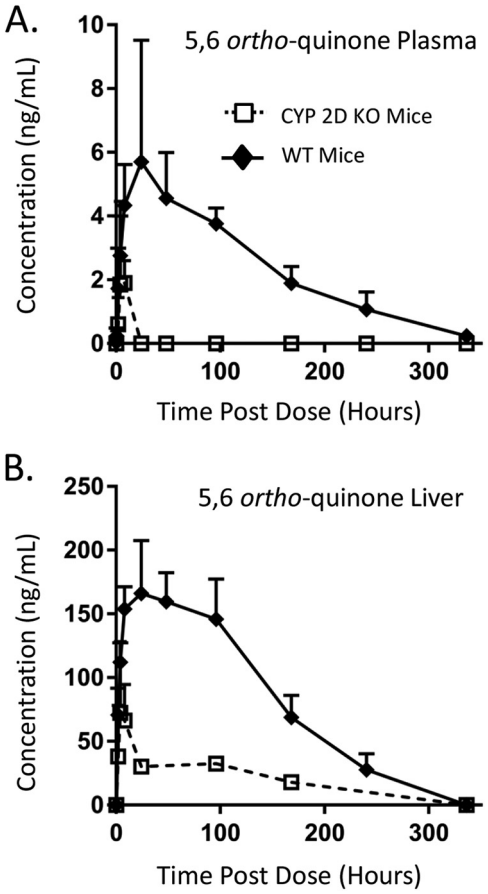

FIG 3 Tafenoquine 5,6-*ortho*-quinone pharmacokinetics in WT C57BL/6 and CYP 2D KO mice. (A) Plasma pharmacokinetic profiles for tafenoquine 5,6-*ortho*-quinone in CYP 2D KO and WT mice. (B) Liver pharmacokinetic profiles for tafenoquine 5,6-*ortho*-quinone in CYP 2D KO and WT mice. The error bars show the standard deviations from replicate analyses (WT, $n = 3$; KO, $n = 4$).

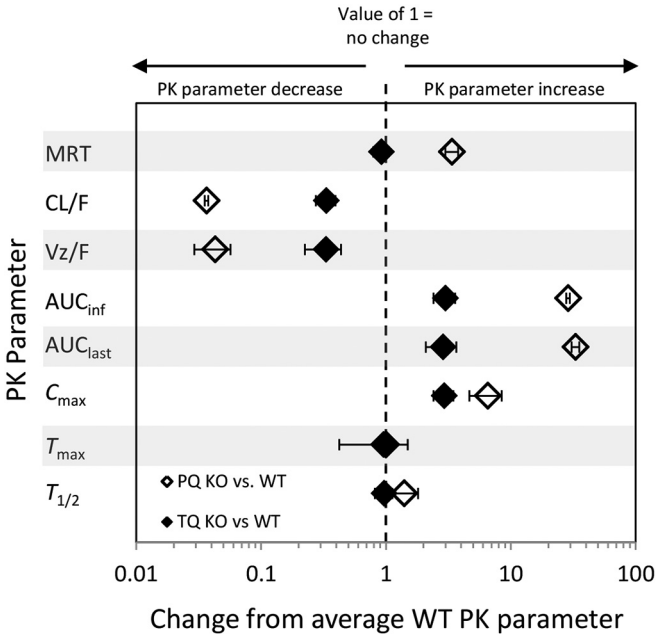

FIG 4 Relative fold changes of primaquine and tafenoquine KO pharmacokinetic parameters from reference (WT) parameters in mouse liver. Indicated are the fold changes of primaquine and tafenoquine pharmacokinetic parameters in the CYP 2D KO strain compared to WT C57BL/6 mice. A value of 1 represents no relative change in the pharmacokinetic parameter between the KO and WT mice. The shading is provided for visual clarity. The error bars show the standard deviations of relative fold changes for KO pharmacokinetic parameters compared to WT mean pharmacokinetic parameter values. The primaquine comparison was conducted using results from Potter et al. (25).

The pharmacokinetic parameters from the profiles shown in Fig. 2B were determined and are indicated in Table 1. The liver half-life of tafenoquine in the KO strain was not statistically significantly different from that in the WT strain (80.1 h for the KO versus 83.5 h for the WT; $P = 0.8$). There was no notable difference in the T_{max} ($P = 0.8$); however, the C_{max} was higher in the KO mice than in the WT mice (85.8 ng/ml for the KO versus 29.3 ng/ml for the WT; $P = 0.002$). The $AUC_{0\text{-inf}}$ value for the KO mice was higher than for the WT mice (10,317.7 h · μg/ml for the KO versus 3,447.9 h · μg/ml for the WT; $P = 0.002$). The CL/*F* value was lower in the KO mice than in the WT mice (2.0 ml/h/kg for the KO versus 6.0 ml/h/kg for the WT; $P = 0.002$). Similar to plasma pharmacokinetic results for tafenoquine, the liver pharmacokinetic parameters from the two strains indicate that decreased CYP 2D metabolism had a systemic effect on the terminal elimination kinetics of tafenoquine, resulting in higher overall exposure of the unmodified parent molecule in KO mouse livers.

Pharmacokinetic evaluation of the tafenoquine 5,6-*ortho*-quinone metabolite. The results presented above indicate that differential CYP 2D metabolism in mice has an effect on the overall pharmacokinetic profile of tafenoquine and primarily alters elimination kinetics *in vivo*. To further investigate the effect of differential CYP 2D metabolism on tafenoquine pharmacokinetics, the presence of a 5,6-*ortho*-quinone metabolite of tafenoquine was probed in both plasma and liver matrices. The pharmacokinetic results obtained for the tafenoquine 5,6-*ortho*-quinone metabolite are shown in Fig. 3. The plasma profile is shown in Fig. 3A and indicates that the metabolite was present in plasma from WT mice and reached a mean maximum concentration of 6.1 ng/ml. A small amount of this metabolite was also detected in plasma from the KO animals, albeit at considerably lower levels. The liver profiles of the tafenoquine 5,6-*ortho*-quinone metabolite are shown in Fig. 3B and indicated trends similar to those in the plasma pharmacokinetic profiles. The metabolite was at the highest abundance in the WT animals, reaching a maximum concentration of 172.6 ng/ml. Interestingly, the half-life of the 5,6-*ortho*-quinone metabolite in the WT species was similar to that of the parent tafenoquine ($\text{tafenoquine}_{\text{liver}}\ t_{1/2} = 83.5$ h; $\text{5,6-}ortho\text{-quinone}_{\text{liver}}\ t_{1/2} = 61.8$ h). The similar half-lives likely resulted from slow release of the 5,6-*ortho*-quinone species *in vivo* from CYP 2D-mediated metabolism of tafenoquine. Additional evidence that supports metabolic release of the 5,6-*ortho*-quinone species from tafenoquine is the significantly shorter half-life and the pharmacokinetic profile of the same 5,6-*ortho*-quinone metabolite when dosed independently (5,6-*ortho*-quinone from tafenoquine [$t_{1/2\ \text{liver}} = 61.8$ h] versus 5,6-*ortho*-quinone dosed independently [$t_{1/2\ \text{liver}} = 4.0$ h]) in WT animals (see the supplemental material). The lower levels of the 5,6-*ortho*-quinone me-

tabolite in the KO animals indicates that these animals were less efficient at metabolizing tafenoquine than the WT animals. Additionally, the lower levels of the 5,6-*ortho*-quinone metabolite in the poor-metabolizer mice indicates that there is likely an additional, less efficient metabolic pathway capable of producing the 5,6-o*rtho*-quinone species. These results indicate possible metabolic switching of tafenoquine phenolic metabolism to another, less efficient cytochrome P450 enzyme(s) in the absence of CYP 2D metabolism.

DISCUSSION

The pharmacokinetic analysis presented above for tafenoquine aids in the understanding of tafenoquine efficacy in the context of CYP 2D6 metabolism/activation. Tafenoquine's long *in vivo* elimination half-life (mouse, ~60 h; human, ~325 h) and *in vitro* stability have complicated in-depth metabolism studies (13, 16). The results presented above provide *in vivo* evidence that tafenoquine pharmacokinetics differ depending on CYP 2D metabolizer status in mice. The changes in pharmacokinetic profiles for tafenoquine in the KO strain versus WT mice were of lower magnitude than what was observed previously for primaquine (25). These differences in the KO versus WT mouse strains for both molecules in the liver are summarized in Fig. 4 as fold changes in PK parameters (KO versus WT). Decreased CYP 2D enzyme activity had no major impact on either the primaquine or tafenoquine half-life or T_{max} in mouse liver. C_{max} values were higher for both primaquine (8.8-fold) and tafenoquine (2.9-fold) in the KO mice than in the WT strain. AUC_{inf} is another parameter that was elevated for both primaquine (34-fold) and tafenoquine (2.9-fold) in the KO strain compared to the WT mice. These changes likely arise from decreased CYP 2D-mediated clearance of both molecules, as indicated by decreased liver CL/*F* parameters for primaquine (25-fold) and tafenoquine (3-fold) in the KO mice compared to the WT strain.

These pharmacokinetic results suggest that all 8-aminoquinoline molecules that are processed by CYP 2D6 for radical curative activity will likely have variable pharmacokinetics in humans. Humans with the poor-metabolizer CYP 2D6 phenotype, who cannot metabolize tafenoquine to an active metabolite(s), will likely have decreased tafenoquine clearance and elevated AUC values compared to individuals of the CYP 2D6 extensive-metabolizer phenotype. A controlled clinical study of tafenoquine pharmacokinetics in human CYP 2D6 poor-, intermediate-, and extensive-metabolizer genotypes/phenotypes is required to confirm these results. Changes in tafenoquine CYP 2D6-mediated clearance could potentially exacerbate any parent molecule-mediated toxicity despite drastically reducing its liver-stage efficacy mediated through CYP 2D6 metabolism. Measurements of drug-associated toxicity were not taken during these pharmacokinetic experiments, and further toxicological studies could be conducted in WT and CYP 2D KO mice to determine if there is a difference due to altered CYP 2D metabolism. The tafenoquine-CYP 2D interaction is also problematic due to the likelihood of CYP 2D6-mediated drug-drug interactions in humans with other antimalarial drugs and commonly prescribed medications. It is currently unknown if these interactions exist and how they would alter efficacy and toxicity.

In addition to parent tafenoquine pharmacokinetics, the formation of a dearylated metabolite was confirmed in plasma and livers of tafenoquine-treated mice. The exact role that the 5,6-*ortho*-quinone species plays in tafenoquine efficacy and/or hemolytic toxicity remains to be determined; however, its presence indicates that mouse CYP 2D metabolism is capable of producing metabolites for tafenoquine similar to those previously reported for primaquine (25). The 5,6-*ortho*-quinone species of tafenoquine would likely be a stable oxidative product of the 5-dearylation pathway. The 5 position of primaquine is open for oxidative modification and differs from that of tafenoquine in that tafenoquine has a 5-*O*-aryl group that prevents the 5 position from direct oxidation. Tafenoquine has been previously reported to undergo oxidative cleavage of the *O*-aryl moiety (32), presumably via arene oxide formation and subsequent hydrolytic cleavage (33). It is likely that human CYP 2D6 also catalyzes the conversion of tafenoquine to the 5,6-*ortho*-quinone species, in addition to producing other phenolic metabolites. One or more of these metabolites are likely capable of redox cycling and generating oxidative stress that is responsible for tafenoquine radical cure activity.

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We declare that we have no competing interests.

REFERENCES

1. **Dow GS, Gettayacamin M, Hansukjariya P, Imerbsin R, Komcharoen S, Sattabongkot J, Kyle D, Milhous W, Cozens S, Kenworthy D, Miller A, Veazey J, Ohrt C.** 2011. Radical curative efficacy of tafenoquine combination regimens in *Plasmodium cynomolgi*-infected Rhesus monkeys (Macaca mulatta). Malar J **10:**212. http://dx.doi.org/10.1186/1475-2875-10-212.
2. **Edgcomb JH, Arnold J, Yount EH, Jr, Alving AS, Eichelberger L, Jeffery GM, Eyles D, Young MD.** 1950. Primaquine, SN 13272, a new curative agent in vivax malaria; a preliminary report. J Natl Malar Soc **9:**285–292.
3. **Llanos-Cuentas A, Lacerda MV, Rueangweerayut R, Krudsood S, Gupta SK, Kochar SK, Arthur P, Chuenchom N, Mohrle JJ, Duparc S, Ugwuegbulam C, Kleim JP, Carter N, Green JA, Kellam L.** 2014. Tafenoquine plus chloroquine for the treatment and relapse prevention of *Plasmodium vivax* malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. Lancet **383:**1049–1058. http://dx.doi.org/10.1016/S0140-6736(13)62568-4.
4. **Walsh DS, Looareesuwan S, Wilairatana P, Heppner DG, Jr, Tang DB, Brewer TG, Chokejindachai W, Viriyavejakul P, Kyle DE, Milhous WK, Schuster BG, Horton J, Braitman DJ, Brueckner RP.** 1999. Randomized dose-ranging study of the safety and efficacy of WR 238605 (Tafenoquine) in the prevention of relapse of *Plasmodium vivax* malaria in Thailand. J Infect Dis **180:**1282–1287. http://dx.doi.org/10.1086/315034.
5. **Tekwani BL, Walker LA.** 2006. 8-Aminoquinolines: future role as antiprotozoal drugs. Curr Opin Infect Dis **19:**623–631. http://dx.doi.org/10.1097/QCO.0b013e328010b848.
6. **Vale N, Moreira R, Gomes P.** 2009. Primaquine revisited six decades after its discovery. Eur J Med Chem **44:**937–953. http://dx.doi.org/10.1016/j.ejmech.2008.08.011.
7. **Bennett JW, Pybus BS, Yadava A, Tosh D, Sousa JC, McCarthy WF, Deye G, Melendez V, Ockenhouse CF.** 2013. Primaquine failure and cytochrome P-450 2D6 in *Plasmodium vivax* malaria. N Engl J Med **369:**1381–1382. http://dx.doi.org/10.1056/NEJMc1301936.
8. **Mihaly GW, Ward SA, Edwards G, Nicholl DD, Orme ML, Brecken-**

ridge AM. 1985. Pharmacokinetics of primaquine in man. I. Studies of the absolute bioavailability and effects of dose size. Br J Clin Pharmacol **19:** 745–750.

9. **Myint HY, Berman J, Walker L, Pybus B, Melendez V, Baird JK, Ohrt C.** 2011. Improving the therapeutic index of 8-aminoquinolines by the use of drug combinations: review of the literature and proposal for future investigations. Am J Trop Med Hyg **85:**1010–1014. http://dx.doi.org/10.4269/ajtmh.2011.11-0498.
10. **Pybus BS, Marcsisin SR, Jin X, Deye G, Sousa JC, Li Q, Caridha D, Zeng Q, Reichard GA, Ockenhouse C, Bennett J, Walker LA, Ohrt C, Melendez V.** 2013. The metabolism of primaquine to its active metabolite is dependent on CYP 2D6. Malar J **12:**212. http://dx.doi.org/10.1186/1475-2875-12-212.
11. **Ward SA, Mihaly GW, Edwards G, Looareesuwan S, Phillips RE, Chanthavanich P, Warrell DA, Orme ML, Breckenridge AM.** 1985. Pharmacokinetics of primaquine in man. II. Comparison of acute vs chronic dosage in Thai subjects. Br J Clin Pharmacol **19:**751–755.
12. **Brueckner RP, Fleckenstein L.** 1991. Simultaneous modeling of the pharmacokinetics and methemoglobin pharmacodynamics of an 8-aminoquinoline candidate antimalarial (WR 238605). Pharm Res **8:**1505–1510. http://dx.doi.org/10.1023/A:1015842316177.
13. **Brueckner RP, Lasseter KC, Lin ET, Schuster BG.** 1998. First-time-in-humans safety and pharmacokinetics of WR 238605, a new antimalarial. Am J Trop Med Hyg **58:**645–649.
14. **Edstein MD, Kocisko DA, Brewer TG, Walsh DS, Eamsila C, Charles BG.** 2001. Population pharmacokinetics of the new antimalarial agent tafenoquine in Thai soldiers. Br J Clin Pharmacol **52:**663–670. http://dx.doi.org/10.1046/j.1365-2125.2001.01482.x.
15. **Kocisko DA, Walsh DS, Eamsila C, Edstein MD.** 2000. Measurement of tafenoquine (WR 238605) in human plasma and venous and capillary blood by high-pressure liquid chromatography. Ther Drug Monit **22:**184–189. http://dx.doi.org/10.1097/00007691-200004000-00008.
16. **Li Q, O'Neil M, Xie L, Caridha D, Zeng Q, Zhang J, Pybus B, Hickman M, Melendez V.** 2014. Assessment of the prophylactic activity and pharmacokinetic profile of oral tafenoquine compared to primaquine for inhibition of liver stage malaria infections. Malar J **13:**141. http://dx.doi.org/10.1186/1475-2875-13-141.
17. **Hale BR, Owusu-Agyei S, Fryauff DJ, Koram KA, Adjuik M, Oduro AR, Prescott WR, Baird JK, Nkrumah F, Ritchie TL, Franke ED, Binka FN, Horton J, Hoffman SL.** 2003. A randomized, double-blind, placebo-controlled, dose-ranging trial of tafenoquine for weekly prophylaxis against *Plasmodium falciparum*. Clin Infect Dis **36:**541–549. http://dx.doi.org/10.1086/367542.
18. **Shanks GD, Oloo AJ, Aleman GM, Ohrt C, Klotz FW, Braitman D, Horton J, Brueckner R.** 2001. A new primaquine analogue, tafenoquine (WR 238605), for prophylaxis against *Plasmodium falciparum* malaria. Clin Infect Dis **33:**1968–1974. http://dx.doi.org/10.1086/324081.
19. **Walsh DS, Eamsila C, Sasiprapha T, Sangkharomya S, Khaewsathien P, Supakalin P, Tang DB, Jarasrumgsichol P, Cherdchu C, Edstein MD, Rieckmann KH, Brewer TG.** 2004. Efficacy of monthly tafenoquine for prophylaxis of *Plasmodium vivax* and multidrug-resistant *P. falciparum* malaria. J Infect Dis **190:**1456–1463. http://dx.doi.org/10.1086/424468.
20. **Nasveld PE, Edstein MD, Reid M, Brennan L, Harris IE, Kitchener SJ, Leggat PA, Pickford P, Kerr C, Ohrt C, Prescott W, Tafenoquine Study Team.** 2010. Randomized, double-blind study of the safety, tolerability, and efficacy of tafenoquine versus mefloquine for malaria prophylaxis in nonimmune subjects. Antimicrob Agents Chemother **54:**792–798. http://dx.doi.org/10.1128/AAC.00354-09.
21. **Dow GS, McCarthy WF, Reid M, Smith B, Tang D, Shanks GD.** 2014. A retrospective analysis of the protective efficacy of tafenoquine and mefloquine as prophylactic anti-malarials in non-immune individuals during deployment to a malaria-endemic area. Malar J **13:**49. http://dx.doi.org/10.1186/1475-2875-13-49.
22. **Marcsisin SR, Sousa JC, Reichard GA, Caridha D, Zeng Q, Roncal N, McNulty R, Careagabarja J, Sciotti RJ, Bennett JW, Zottig VE, Deye G, Li Q, Read L, Hickman M, Dhammika Nanayakkara NP, Walker LA, Smith B, Melendez V, Pybus BS.** 2014. Tafenoquine and NPC-1161B require CYP 2D metabolism for anti-malarial activity: implications for the 8-aminoquinoline class of anti-malarial compounds. Malar J **13:**2. http://dx.doi.org/10.1186/1475-2875-13-2.
23. **Pybus BS, Sousa JC, Jin X, Ferguson JA, Christian RE, Barnhart R, Vuong C, Sciotti RJ, Reichard GA, Kozar MP, Walker LA, Ohrt C, Melendez V.** 2012. CYP450 phenotyping and accurate mass identification of metabolites of the 8-aminoquinoline, anti-malarial drug primaquine. Malar J **11:**259. http://dx.doi.org/10.1186/1475-2875-11-259.
24. **Miller AK, Harrell E, Ye L, Baptiste-Brown S, Kleim JP, Ohrt C, Duparc S, Mohrle JJ, Webster A, Stinnett S, Hughes A, Griffith S, Beelen AP.** 2013. Pharmacokinetic interactions and safety evaluations of coadministered tafenoquine and chloroquine in healthy subjects. Br J Clin Pharmacol **76:**858–867. http://dx.doi.org/10.1111/bcp.12160.
25. **Potter BM, Xie LH, Vuong CT, Zhang J, Zhang P, Duan D, Luong T-LT, Bandara Herath HMT, Dhammika Nanayakkara NP, Tekwani BL, Walker LA, Nolan CK, Sciotti RJ, Zottig VE, Smith PL, Paris RM, Read LT, Li Q, Pybus BS, Sousa JC, Reichard GA, Marcsisin SR.** 2015. Differential CYP 2D6 metabolism alters primaquine pharmacokinetics. Antimicrob Agents Chemother **59:**2380–2387. http://dx.doi.org/10.1128/AAC.00015-15.
26. **Scheer N, Kapelyukh Y, McEwan J, Beuger V, Stanley LA, Rode A, Wolf CR.** 2012. Modeling human cytochrome P450 2D6 metabolism and drug-drug interaction by a novel panel of knockout and humanized mouse lines. Mol Pharmacol **81:**63–72. http://dx.doi.org/10.1124/mol.111.075192.
27. **Yu AM, Haining RL.** 2006. Expression, purification, and characterization of mouse CYP2d22. Drug Metab Dispos **34:**1167–1174. http://dx.doi.org/10.1124/dmd.105.008870.
28. **Bowman ZS, Oatis JE, Jr, Whelan JL, Jollow DJ, McMillan DC.** 2004. Primaquine-induced hemolytic anemia: susceptibility of normal versus glutathione-depleted rat erythrocytes to 5-hydroxyprimaquine. J Pharmacol Exp Ther **309:**79–85. http://dx.doi.org/10.1124/jpet.103.062984.
29. **Vasquez-Vivar J, Augusto O.** 1990. ESR detection of free radical intermediates during autoxidation of 5-hydroxyprimaquine. Free Radic Res Commun **9:**383–389. http://dx.doi.org/10.3109/10715769009145698.
30. **Vasquez-Vivar J, Augusto O.** 1992. Hydroxylated metabolites of the antimalarial drug primaquine. Oxidation and redox cycling. J Biol Chem **267:**6848–6854.
31. **Vasquez-Vivar J, Augusto O.** 1994. Oxidative activity of primaquine metabolites on rat erythrocytes in vitro and in vivo. Biochem Pharmacol **47:**309–316. http://dx.doi.org/10.1016/0006-2952(94)90022-1.
32. **Idowu OR, Peggins JO, Brewer TG, Kelley C.** 1995. Metabolism of a candidate 8-aminoquinoline antimalarial agent, WR 238605, by rat liver microsomes. Drug Metab Dispos **23:**1–17.
33. **O'Neill P, Tingle MD, Mahmud R, Storr RC, Ward SA, Kevin Park B.** 1995. The effect of fluorine substitution on the haemotoxicity of primaquine. Bioorg Med Chem Lett **5:**2309–2314. http://dx.doi.org/10.1016/0960-894X(95)00400-N.