PFMDR1 POLYMORPHISMS INFLUENCE ON *IN VITRO* SENSITIVITY OF THAI *PLASMODIUM FALCIPARUM* ISOLATES TO PRIMAQUINE, SITAMAQUINE AND TAFENOQUINE

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Abstract. Primaquine (PQ), an 8-aminoquinoline, is considered a tissue schizonticide drug for radical cure in vivax and ovale malaria, with minimal impact on asexual erythrocytic stages at therapeutic concentrations. Tafenoquine (TQ), a new 8-aminoquinoline analog of PQ, is active against both malaria parasite tissue and blood stages and is being promoted as a drug candidate for antimalarial chemotherapy and chemoprophylaxis and potential transmission blocking against *Plasmodium vivax* and *P. falciparum*. This study compared *in vitro* sensitivity of Thai *P. falciparum* isolates against three 8-aminoquinolines, PQ, TQ and sitamaquine (SQ), a related 8-aminoquinoline and assessed the importance of *pfmdr1* polymorphism on the *in vitro* response. Seventy-eight laboratory adapted Thai *P. falciparum* isolates were evaluated for *in vitro* sensitivity to the three 8-aminoquinolines using a radioisotopic assay, and *pfmdr1* polymorphisms were determined using PCR-based methods. All three drugs have weak antiplasmodial activity against asexual erythrocytic stage with SQ being the most potent by almost 10 folds. Cross susceptibility was observed in all three 8-aminoquinolines. Parasites containing *pfmdr1* 86Y, 184Y or 1034S allele exhibit significantly higher PQ IC_{50}. TQ sensitivity was reduced in those parasites containing *pfmdr1* 86Y, 1034S or 1042N allele. However, there was no significant influence of *pfmdr1* alleles on SQ sensitivity. The data highlight unique differences among three representative 8-aminoquinoline drugs that may be useful in understanding their potential utility in antimalarial development.

Keywords: *Plasmodium falciparum*, *in vitro* sensitivity, *pfmdr1*, primaquine, sitamaquine, tafenoquine, Thailand

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INTRODUCTION

There is an urgent need for new and effective antimalarial drugs, partly to address the issue of parasite resistance to existing drugs (a serious issue across all of Southeast Asia) (Dondorp *et al*, 2009; WHO, 2010; Witkowski *et al*, 2013;

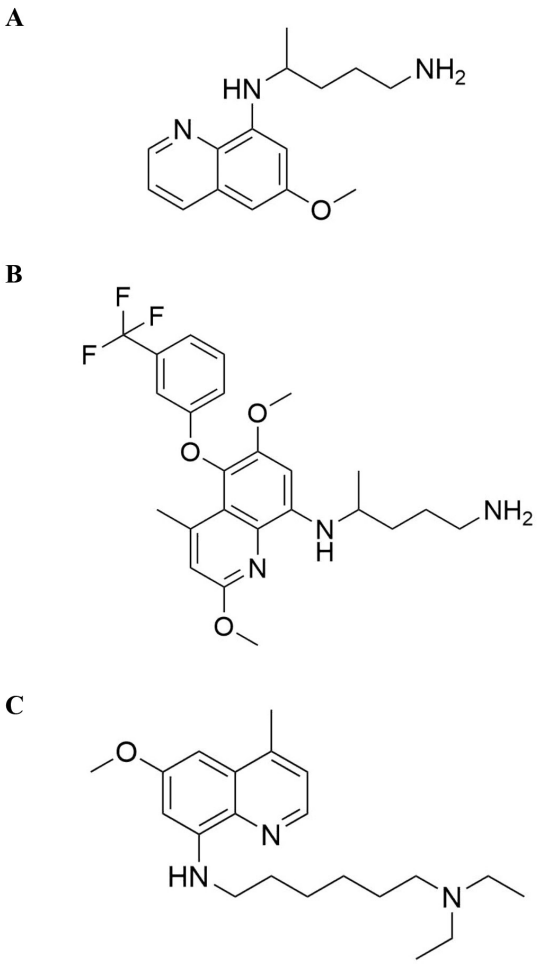

Fig 1–Chemical structures of primaquine (A), tafenoquine (B) and sitamaquine (C).

Mungthin *et al*, 2014) and also to meet demands of a malaria eradication agenda, which requires drugs that can deliver radical cure and transmission blocking activities (Graves *et al*, 2015).

Primaquine (PQ) is an 8-aminoquinoline **(**Fig 1) and is the only clinically available tissue schizonticide drug for radical cure in vivax and ovale malaria (WHO, 2006). PQ also has transmission-blocking activity for falciparum malaria due to its gametocytocidal activity and is currently under intense investigation as a tool for eradication of falciparum malaria (Graves *et al*, 2015). However, PQ is not considered a blood schizonticide as it shows little activity against asexual erythrocytic stages at therapeutically achievable and safe drug exposure (Vennerstrom *et al*, 1999; Bray *et al*, 2005; Pradines *et al*, 2006). Tafenoquine (TQ) is a related 8-aminoquinoline (Fig 1) currently under clinical phase III development for prophylaxis and treatment of human malaria (Dow *et al*, 2014; Llanos-Cuentas *et al*, 2014). It is argued that TQ has activity against blood stages of a range of malaria parasites including *Plasmodium falciparum* and *P. vivax* at therapeutically relevant exposure (Peters, 1999). In addition, its gametocytocidal action may also contribute to a reduction of transmission. However, the major drawback for worldwide use is that, like PQ, TQ can cause hemolytic anemia in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency (von Seidlein *et al*, 2013). Although another 8-aminoquinoline analog, sitamaquine (SQ) (Fig 1), was developed for the treatment of leishmaniasis (Jha *et al*, 2005; Wasunna *et al*, 2005), there is little information on SQ activity against *Plasmodium* spp especially multidrug-resistant strains.

The antiplasmodial mechanism of action of 8-aminoquinolines remains unclear. Proposed mechanisms include inhibition of hemazoin formation, interference with mitochondrial electron transport and/or increasing oxidant stress (Vennerstrom and Eaton, 1988; Ittarat *et al*, 1994; Vennerstrom *et al*, 1999). Antimalarial activity of PQ is likely to result from reactive metabolites that may become oxidized and initiate radical formation and reactive oxygen species (Fletcher *et al*, 1988; Vennerstrom and Eaton, 1988). Both PQ and TQ require metabolism by human

CYP 2D enzymes for their antimalarial activity (Pybus *et al*, 2012, 2013; Marcsisin *et al*, 2014). Because PQ and TQ are thought to act through their reactive metabolites, their antimalarial activities might not be well correlated with the values generated by *in vitro* tests (Pybus *et al*, 2012, 2013; Marcsisin *et al*, 2014).

Resistance to quinoline-based antimalarials has been linked to a number of genes. Most notably a point mutation in *P. falciparum* chloroquine resistance transporter gene (*pfcrt*) resulting in K76T mutation has been linked to resistance to the 4-aminoquinoline, chloroquine (CQ), in *P. falciparum* isolates (Fidock *et al*, 2000; Cooper *et al*, 2002). In addition, the level of *in vitro* CQ resistance can be modulated by *P. falciparum* multidrug resistance 1 gene (*pfmdr1*) (Babiker *et al*, 2001; Setthaudom *et al*, 2011). The *pfmdr1*, located on chromosome 5, encodes a P-glycoprotein homologue 1 (Pgh1) (Foote *et al*, 1990). At least 5 mutations on the *pfmdr1* gene have been identified, *viz*, N86Y, Y184F, S1034C, N1042D and D1246Y (Wilson *et al*, 1993). In addition, a number of studies have shown that mutations and amplification of *pfmdr1* also are associated with *in vitro* and *in vivo* resistance to mefloquine (MQ), an arylaminoalcohol (Cowman *et al*, 1994; Duraisingh *et al*, 2000a; Pickard *et al*, 2003; Price *et al*, 2004).

There is compelling evidence supporting a role for *pfmdr1* in the *in vitro* response to other antimalarials, such as quinine (QN) and lumefantrine (LF) and artemisinin derivatives (Duraisingh *et al*, 2000b; Mungthin *et al*, 2010; Poyomtip *et al*, 2012). As *P. falciparum* isolated from different areas in Thailand exhibit different *pfmdr1* polymorphisms and antimalarial resistance phenotypic patterns (Mungthin *et al*, 2014), new drugs or drug combinations should carefully be evaluated in a wide range of parasite strains with different phenotypes and genotypes before they can be considered before proceeding with further development as both chemotherapy and chemoprophylaxis. The present study determined the range of *in vitro* sensitivities to the 8-aminoquinolines (PQ, SQ and TQ) of a large collection of laboratory adapted *P. falciparum* isolates collected from different geographical areas across Thailand and accessed the influence of *pfmdr1* on *in vitro* drug sensitivity.

MATERIALS AND METHODS

P. falciparum isolates

Eighty-one isolates of *P. falciparum* including three standard laboratory isolates (K1, M12, and 3D7) and 78 newer Thai isolates were investigated. The 78 isolates of *P. falciparum* were obtained from patients with uncomplicated falciparum malaria who attended malaria clinics and hospitals in malaria endemic areas along the Thailand-Myanmar and Thailand-Cambodia borders from 2003 to 2013. Parasites were maintained in continuous culture using a modification of the candle-jar method of Trager and Jensen (1976) in human erythrocytes (O^+) at 37°C and RPMI 1640 medium supplemented with 23 mM $NaHCO_3$, 25 mM N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) and 10% human AB serum under an atmosphere of 90% N_2, 5% O_2 and 5% CO_2.

The research protocol was reviewed and approved by the Ethics Committee of the Royal Thai Army Medical Department (IRBRTA0764/2558).

Antimalarial *in vitro* inhibition assay

PQ, SQ and TQ sensitivity of *P. falciparum* isolates was determined by measurement of [^{3}H]hypoxanthine in-

corporation into parasite nucleic acids as previously described (Desjardins *et al*, 1979). Drug IC_{50} (concentration of drug inhibiting parasite growth by 50%) was determined from log_{10} dose/response relationship using GRAFIT (Erithacus Software, Kent, England) curve fitting software. PQ, TQ and SQ IC_{50s} of each isolate are recorded as the mean IC_{50} of three independent experiments carried out in triplicate.

Analysis of *pfcrt* and *pfmdr1* mutations and *pfmdr1* copy number

Parasite DNA was extracted using Chelex-resin method (Wooden *et al*, 1992). In order to detect *pfcrt* K76T mutation allele-specific restriction PCR was performed in a 25-µl mixture consisting of 1.5 mM $MgCl_2$, 200 µM each dNTP (Pharmacia Biotech, Buckinghamshire, UK), 20 mM Tris-HCl, 0.1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, 50% glycerol, 0.5% Tween®, 0.5% Nonidet® P-40, 2 U *Taq* DNA polymerase (Promega, Ashford, UK), 0.25 µM each specific primer and 2 µl of template DNA. Primers TCRP1 and TCRP2 were used to amplify a 537-bp product flanking the K76T mutation, followed by a secondary PCR using primers TCRD1 and TCRD2 to amplify a 134-bp fragment (Table 1). The resultant amplicon was digested with 1 U *ApoI* at 37°C overnight to cleave amplicon containing 76T (Djimdé *et al*, 2001). Genomic DNA extracted from *P. falciparum* K1, CQ-resistant and 3D7, CQ-sensitive strain were used as positive controls.

Mutations in *pfmdr1* were determined by nested-PCR and restriction endonuclease digestion. All PCR reactions were carried out in 25-µl mixture containing 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8), 2 mM $MgCl_2$, 200 µM each dNTP, 0.25 µM each specific primer (Table 1), 1 U *Taq* DNA polymerase, and 2 µl of template DNA. The primer sequences are described in Table 1. For primary PCR amplification, primers A1 and A3, O1 and O2 yielded amplicons of 657 and 887 bp, respectively. Then primers A2 and A4, 1034f and 1042r, 1246f and O2 were used for the secondary PCR amplification generating amplicons of 560, 233 and 345 bp, respectively. Restriction sites were generated by polymorphisms at codon 86 (for *ApoI* and *AflIII* for 86N and 86Y, respectively), at codon 184 (*DraI* for 184F), at codon 1034 (*DdeI* for 1034S), at codon 1042 (*VspI* forv1042N), and at codon 1246 (*DpnII* and *EcoRV* for 1246N and 1246Y, respectively) (Duraisingh *et al*, 2000a). *P. falciparum* K1 strain was used as a control for the detection of *pfmdr1* N86Y mutation, and 7G8 strain as a control for *pfmdr1* 184F, 1034C, 1042D and 1246Y mutations. Amplicons were analyzed by gel-electrophoresis and the presence of both undigested and digested fragments are considered mixed alleles.

The *pfmdr1* copy number was determined using TaqMan real-time PCR (ABI sequence detector 7000; Applied Biosystems, Foster City, CA) using primers and probes as shown in Table 1 (Price *et al*, 2004). *P. falciparum* K1 strain and Dd2 clone containing 1 copy and 4 *pfmdr1* copies, respectively were used as reference samples.

Statistical analysis

Data were analyzed using STATA/MP, Version 12 (Stata Corp, College Station, TX). Normally distributed IC_{50} data were assessed by the Kolmogorov-Smirnov test. Differences among parasites with different genotypes were analyzed by chi-square and Fisher's exact test. Differences in the mean PQ, TQ and SQ IC_{50} among parasites from different groups were analyzed by independent *t*-test or one-way ANOVA. Post

Table 1
Primers and probes used for the detection of *pfcrt* and *pfmdr1* polymorphisms.

Gene	Primer	Sequence
pfcrt	TCRP1	5′ CCGTTAATAATAAATACACGCAG 3′
	TCRP2	5′ CGGATGTTACAAAACTATAGTTACC 3′
	TCRD1	5′ TGTGCTCATGTGTTTAAACTT 3′
	TCRD2	5′ CAAAACTATAGTTACCAATTTTG 3′
pfmdr1	A1	5′ TGTTGAAAGATGGGTAAAGAGCAGAAAGAG 3′
	A3	5′ TACTTTCTTATTACATATGACACCACAAACA 3′
	A2	5′ GTCAAACGTGCATTTTTTATTAATGACCATTA 3′
	A4	5′ AAAGATGGTAACCTCAGTATCAAAGAAGAG 3′
	O1	5′ AGAAGATTATTTCTGTAATTTGATACAAAAAGC 3′
	O2	5′ ATGATTCGATAAATTCATCTATAGCAGCAA 3′
	1034f	5′AGAATTATTGTAAATGCAGCTTTATGGGGACTC 3′
	1042r	5′AATGGATAATATTTCTCAAATGATAACTTAGCA 3′
	1246f	5′ ATGATCACATTATATTAAAAAATGATATGACAAAT 3′
pfmdr1 copy number	*pfmdr1*-1F	5′ TGCATCTATAAAACGATCAGACAAA 3′
	pfmdr1-1R	5′ TCGTGTGTTCCATGTGACTGT 3′
	pfmdr1-probe	5′ TTTAATAACCCTGATCGAAATGGAACCTTTG 3′
	β-tubulin-1F[a]	5′ TGATGTGCGCAAGTGATCC 3′
	β-tubulin-1R	5′ TCCTTTGTGGACATTCTTCCTC 3′
	β-tubulin-probe	5′ TAGCACATGCCGTTAAATATCTTCCATGTCT 3'

[a]Internal control gene.

Hoc test (Scheffe) for multiple comparisons was used to test for differences among groups. The level of significance was set at a *p*-value of < 0.05. Cross susceptibility across the three 8-animoquinolines was analyzed by Pearson's correlation.

RESULTS

Seventy-eight *P. falciparum* isolates from areas along the Thailand-Myanmar and Thailand-Cambodia borders from 2003 to 2013 were tested for *in vitro* sensitivity to PQ, TQ and SQ. The mean $IC_{50} \pm$ SD for PQ, TQ and SQ was 3.3 ± 1.2 (range 1.0 to 7.5), 2.5 ± 1.2 (range 0.4 to 6.1) and 0.3 ± 0.1 (range 0.1 to 0.6) μM, respectively (Table 2). PQ, TQ and SQ IC_{50} values in this population of isolates are normally distributed. Drug susceptibility across all three drugs correlates significantly: PQ IC_{50} correlates positively with TQ IC_{50} ($r = 0.447$, $p < 0.001$) and SQ IC_{50} ($r = 0.542$, $p < 0.001$), and TQ IC_{50} correlates positively with SQ IC_{50} ($r = 0.547$, $p < 0.001$).

When the parasite isolates were analyzed for mutations in *pfcrt* and *pfmdr1*, all isolates contained CQ-resistant genotype *pfcrt* 76T, while *pfmdr1* 184F allele was more common in parasites isolated from Thailand-Cambodia than those from Thailand-Myanmar border regions (χ^2, $p < 0.001$) and no isolates contained mutation at codon 1246 (Table 3). The laboratory isolates, K1 and M12, contained *pfmdr1* 86N allele while 3D7 contained *pfmdr1* 184F allele. Mean ± SD *pfmdr1* gene copy number of the field isolates was 2.1 ± 1.0 (range 0.6 to 4.7), with significantly higher copy number present in parasites from Thailand-Myanmar than Thailand-

Table 2
In vitro sensitivity to PQ, SQ and TQ and distribution of *pfmdr1* polymorphisms of Thai *P. falciparum* isolates collected during 2003-2013.

Region	No. of isolates	PQ IC_{50} (μM)	TQ IC_{50} (μM)	SQ IC_{50} (μM)	*pfmdr1* copy number	*pfmdr1* allele				
						86Y	184F	1034C	1042D	1246Y
Thailand-Myanmar	52	3.3 ± 1.1[a]	2.4 ± 1.2	0.3 ± 0.1	2.5 ± 0.9	7 (13%)	17 (33%)	5 (10%)	5 (10%)	-
Thailand-Cambodia	26	3.1 ± 1.3	2.5 ± 1.1	0.3 ± 0.1	1.3 ± 0.8	3 (11%)	22 (85%)	3 (11%)	6 (23%)	-
Total	78	3.3 ± 1.2	2.4 ± 1.1	0.3 ± 0.1	2.1 ± 1.0	10 (13%)	39 (50%)	8 (10%)	11 (14%)	-

[a]Mean ± SD.
PQ, primaquine; SQ, sitamaquine; TQ, tafenoquine.

Cambodia border ($p < 0.001$).

Parasites containing *pfmdr1* 86Y, 184Y or 1034S allele exhibit significantly higher PQ IC_{50} values, whereas reduced TQ sensitivity was seen in parasites containing 86Y, 1034S or 1042N allele (Table 3). However, *pfmdr1* allele types had no effect on SQ sensitivity. *pfmdr1* copy number (from 1 to 4 copies) had no impact on 8-aminoquinoline drug susceptibility. When classified according to *pfmdr1* haplotypes, significant differences in PQ and TQ IC_{50} values are observed among these haplotype groups (one-way ANOVA, $p = 0.004$ and 0.021, respectively) (Table 4). Multiple comparison indicated that parasites carrying *pfmdr1* 86Y allele are significantly less sensitive to PQ than those with 184F or 1042D ($p = 0.021$ and 0.025, respectively); those containing 86Y allele significantly higher TQ IC_{50} value than with 1042D ($p = 0.030$); and no specific *pfmdr1* haplotype was associated with SQ sensitivity.

DISCUSSION

In this study, we report the sensitivities of 8-aminoquinolines, PQ, SQ and TQ against 78 Thai isolates of *P. falciparum* collected at the Thailand-Myanmar and Thailand-Cambodia border areas during 2003 - 2013 and 3 laboratory strains. This is the first report of SQ sensitivity in multidrug-resistant Thai *P. falciparum* isolates. SQ, an antileishmanial agent that has progressed to phase II clinical trials for the treatment of visceral leishmaniasis (Jha *et al*, 2005; Wasunna *et al*, 2005), showed superior *in vitro* sensitivity compared to PQ and TQ (approximately 10- and 7-fold more active, respectively). The PQ and TQ IC_{50} values of Thai isolates were in a similar range with previously published reports (Vennerstrom *et al*, 1999; Bray *et al*, 2005; Pradines *et al*, 2006). It is unclear if

Table 3
Comparison of PQ, TQ and SQ sensitivity among Thai *P. falciparum* isolates with different *pfmdr1* alleles collected at Thailand-Myanmar and Thailand-Cambodia border regions during 2003-2013.

pfmdr1 allele		No.	PQ IC_{50} (μM)	*p*-value	TQ IC_{50} (μM)	*p*-value	SQ IC_{50} (μM)	*p*-value
86	N	69	3.1 ± 1.1[a]	0.005	2.4 ± 1.1	0.021	0.3 ± 0.1	0.243
	Y	12	4.2 ± 1.5		3.2 ± 1.3		0.4 ± 0.1	
184	Y	42	3.6 ± 1.1	0.039	2.5 ± 1.3	0.688	0.3 ± 0.1	0.561
	F	39	3.0 ± 1.3		2.4 ± 1.1		0.3 ± 0.1	
1034	S	73	3.4 ± 1.2	0.041	2.6 ± 1.2	0.038	0.3 ± 0.1	0.069
	C	8	2.5 ± 1.0		1.6 ± 0.9		0.2 ± 0.1	
1042	N	70	3.4 ± 1.2	0.078	2.6 ± 1.2	0.026	0.3 ± 0.1	0.257
	D	11	2.7 ± 1.1		1.7 ± 0.9		0.3 ± 0.1	
Copy no.	< 3	60	3.4 ± 1.3	0.324	2.4 ± 1.2	0.455	0.3 ± 0.1	0.288
	≥ 3	21	3.1 ± 0.8		2.7 ± 1.3		0.4 ± 0.1	

[a]Mean ± SD.
PQ, primaquine; SQ, sitamaquine; TQ, tafenoquine.

Table 4
Comparison of *in vitro* PQ, TQ and SQ sensitivity among *P. falciparum* isolates with different *pfmdr1* haplotypes collected at Thailand-Myanmar and Thailand-Cambodia border regions during 2003-2013.

Group	*pfmdr1* haplotype				No.	PQ* IC_{50} (μM)	TQ** IC_{50} (μM)	SQ IC_{50} (μM)
	86N/Y	184Y/F	1034S/C	1042N/D				
1	Y	Y	S	N	12	4.2 ± 1.5[a]	3.2 ± 1.3	0.4 ± 0.1
2	N	F	S	N	28	2.9 ± 1.1	2.6 ± 1.1	0.3 ± 0.1
3	N	Y/F	S/C	D	11	2.7 ± 1.1	1.7 ± 0.9	0.3 ± 0.1
4	N	Y	S	N	30	3.5 ± 1.0	2.3 ± 1.2	0.3 ± 0.1

[a]Mean ± SD.
*$p = 0.004$, **$p = 0.021$ among groups determined by one-way ANOVA.
PQ, primaquine; SQ, sitamaquine; TQ, tafenoquine.

these micromolar drug sensitivities would have any real impact on parasite viability in an *in vivo* context where peak drug concentrations for PQ and TQ are in the 100 and 400 ng/ml range, respectively (Mihaly *et al*, 1984; Ward *et al*, 1985; Brueckner *et al*, 1998). It is also unclear if sub-optimal drug exposure of asexual stages could drive acquisition of resistance, which could ultimately impact on sensitivity of liver or gametocyte stages of the parasite life cycle. There was no difference in PQ, SQ and TQ IC_{50} values between parasites from Thailand-Myanmar and Thailand-Cambodia border areas. The problem of antimalarial drug resistance is complicated by cross resistance, in which resistance to one drug confers resistance to other

drugs in the same chemical family or sharing similar modes of action. There was no correlation between parasite sensitivity to these three 8-aminoquinolines and the currently used quinolines in Thailand, namely, CQ, MQ or QN IC_{50} (data not shown), although there was a clear correlation in parasite susceptibility among the three drugs. The present study confirms the correlation between PQ and TQ IC_{50} values previously reported (Vennerstrom *et al*, 1999).

In Thailand, PQ has been used for the treatment of vivax malaria for more than 60 years (Baird and Hoffman, 2004). A 14-day course of PQ after CQ treatment is used for eradication of liver hypnozoite stages. This long PQ course may be associated with poor compliance in some patients. In contrast to PQ, TQ has a longer half-life (~14 days versus 0.5 day for PQ) and a similar stage specificity of action, which makes TQ a promising drug to replace PQ, although both drugs retain the potential to cause life threatening hemolysis in G6PD-deficient patients (von Seidlein *et al*, 2013). A recent study showed that a combination of CQ and a single dose of TQ is more effective than CQ alone for treating and preventing relapse in vivax malaria (Llanos-Cuentas *et al*, 2014). TQ has been also shown to be an effective chemoprophylaxis for both vivax and falciparum malaria (Shanks *et al*, 2001; Walsh *et al*, 2004). It is unclear how readily parasites can acquire resistance to 8-aminoquinolines or if there are important differences between parasite stages. It has been possible to select PQ resistance in *P. vivax* using a drug pressure protocol (Arnold *et al*, 1961). In Thailand, vivax malaria shares the same distribution as falciparum malaria. Thus, drug treatment for vivax malaria could exert unexpected drug pressure against *P. falciparum* and vice versa. Compared to PQ, TQ has a significantly longer *in vivo* half-life, a property with the propensity for development of parasite drug resistance. TQ resistance was shown to be stable after withdrawal of drug pressure (Peters *et al*, 2003). The impact of such changes in susceptibility on gametocytocidal activity remains to be assessed.

In Thailand, *P. falciparum* isolates from different areas contain different patterns of *pfmdr1* polymorphism and expression, which confer a diverse pattern of drug resistance to a number of antimalarials, in particular the quinolines (Mungthin *et al*, 2014). The present study is the first to show the influence of *pfmdr1* polymorphism on *in vitro* PQ and TQ sensitivity. Parasites containing *pfmdr1* 86Y, 184Y or 1034S allele have significantly higher PQ IC_{50} values, while those with 86Y, 1034S or 1042N allele are significantly less susceptible to TQ. When the parasites were categorized according to their *pfmdr1* haplotypes, it was confirmed that parasites containing the *pfmdr1* 86Y allele were less sensitive to both PQ and TQ. This association is the first to be reported; however, how Pgh1, a protein encoded by *pfmdr1*, modulates the activities of these 8-aminoquinoline is not clear. It has been suggested that Pgh1 might act as a transporter, importing drugs into the parasite digestive vacuole (Rohrbach *et al*, 2006) and mutations in *pfmdr1* might affect this activity. There was no association between the *pfmdr1* copy number and *in vitro* response to PQ, SQ and TQ, and *pfmdr1* polymorphisms had no influence on the SQ IC_{50} values. This finding would agree with data showing that SQ is not a substrate of LMDR1, a P-glycoprotein-like transporter in the ATP-binding cassette (ABC) family conferring miltefosine resistance in *Leishmania tropica* (Pérez-Victoria *et al*, 2011).

G6PD deficiency is one of the most common genetic disorders in humans, being prevalent throughout Africa, Asia and parts of Europe and South America, wherever malaria is or was endemic (Howes *et al*, 2013). Whether SQ has hemolytic toxicity as seen with antimalarial 8-aminoquinolines, PQ and TQ, will be very important for its further evaluation.

The efficacy of PQ has historically been linked to CYP-mediated metabolism. Although to date, no clear evidence exists in the literature, which unambiguously assigns a metabolic pathway or specific metabolites necessary for activity, recent literature suggests a role for CYP 2D6 in the generation redox active metabolites (Pybus *et al*, 2012, 2013; Marcsisin *et al*, 2014). Therefore, CYP 2D6 deficiency patients will be at risk with treatment of 8-aminoquinoline derivatives.

In conclusion, this study shows the baseline *in vitro* sensitivity of Thai *P. falciparum* isolates to the 8-aminoquinolines PQ, SQ and TQ, with SQ the most active. Although cross resistance has been found among these three 8-aminoquinolines, only SQ was not influenced by *pfmdr1* polymorphisms. This information will be useful for rationale development of new antimalarial compounds for both chemotherapy and chemoprophylaxis.

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