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Effect of artesunate and mefloquine in combination on the Fridericia corrected QT intervals in *Plasmodium falciparum* infected adults from Thailand

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Summary

OBJECTIVE To ascertain whether mefloquine (MQ) produces electrocardiogram (ECG) changes that could be a risk for Torsades de Pointe (TdP), a potentially malignant, ventricular tachyarrhythmia.

METHODS We measured the Fridericia corrected QT (QTcF) intervals on 12 lead ECGs on days (D) 0, 3, 7 in *Plasmodium falciparum* infected adults, treated with oral artesunate (AS) and MQ as a new fixed dose (n = 25) combination or loose tablets (n = 25) over 3 days. Target total doses were 12 mg/kg of AS and 24–25 mg/kg of MQ. MQ concentrations ([MQ]) were measured by HPLC.

RESULTS All ECG intervals were similar between drug arms and were combined for analysis. Mean QTcF values were 389 (D0), 407 (D3) and 399 (D7) ms (Ps < 0.003 *vs.* D0); corresponding heart rates and [MQ]s were 83, 67 and 73 beats/minute (Ps≤0.0003 *vs.* D0) and 0, 3095 and 1721 ng/ml. One male patient (loose arm) had a D3 QTcF 504 ms (D0 406 ms, D7 433 ms). In the modelling of QTcF and JTcF from D0 to D7, significant effects were observed individually for [MQ], temperature and heart rate (HR). The MQ $AUC_{0-\infty}$ was not a significant factor. Using a manual descending, model building approach to select variables, the HR was the only significant variable (P = 0.001) over time in the model that best explained the changes in the QTcF and JTcF intervals.

CONCLUSIONS In this small group of patients, slowing heart rates due to malaria resolution best explained the observed increases in the QTcF intervals.

keywords electrocardiogram, QT interval, Torsades de Pointe, mefloquine, artesunate, malaria

Introduction

Drug regulatory authorities demand the assessment of drugs for their potential to cause Torsades de Pointe (TdP), a broad complex, ventricular tachycardia that may lead to ventricular fibrillation and sudden death (Dessertenne 1966). Measuring the corrected QT (QTc) interval on an electrocardiogram (ECG) is recommended but several factors affect its length, including gender, diurnal variation, eating food, body temperature, heart rate (HR), hypokalaemia and hypocalcaemia (White 2007). The risk of TdP and QTc prolongation is not linear and there are many QT correction formulae to choose from (Yap & Camm 2003), including a new QT correction formula derived from malaria patients (Price *et al.* 1998). QT dispersion, advocated previously, is a poor risk marker (Batchvarov & Malik 2000). Seldom mentioned is the JT interval, the ECG measurement of ventricular repolarisation. The regulatory guidelines [International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH guidelines)] acknowledge such challenges and recommend using the Bazett (QTcB) and Fridericia (QTcF) corrected QT data (http://www.ich.org, accessed November 2009).

Ventricular repolarisation is mediated mainly by the outward flow of potassium (K^+) ions from myocytes

through the slow (I_{Ks}) and rapid (I_{Kr}) components of the delayed rectifier K^+ channel. Inhibiting K^+ outflow leads to heterogeneous repolarisation, an environment favouring the development of TdP. The I_{Kr} channel, encoded by the human ether a go go (hERG) gene on chromosome 7, is the target of a broad range of drugs with different chemical structures e.g. halofantrine, cisapride, erythromycin, chlorpromazine, astemizole and quinidine (Curran *et al.* 1995; Sanguinetti *et al.* 1995).

Halofantrine, a potent I_{Kr} inhibitor, causes a dose dependent QTc interval increase and ventricular fibrillation in malaria patients; a problem that was detected only after registration (Monlun *et al.* 1995; Touze *et al.* 1996; Gundersen *et al.* 1997; Malvy *et al.* 2000; Mbai *et al.* 2002). Increases in the mean QTc intervals (up to 20 ms) have been observed in recovering malaria patients with oral artesunate (AS), amodiaquine, sulphadoxine/pyrimethamine (S/P), and dihydroartemisinin/piperaquine on around day (D) 3 when patients are afebrile and have reduced heart rates (Ribeiro & Olliaro 1998; Ngouesse *et al.* 2001; Mytton *et al.* 2007). By contrast, one study found a decrease in the mean D3 QTc interval and mean D3 HR in patients treated with atovaquone proguanil alone or combined with oral AS (Gupta *et al.* 2005).

Mefloquine (MQ) has a good cardiac record (ter Kuile *et al.* 1995; Jaspers *et al.* 1996). Case reports (Fonteyne *et al.* 1996; Richter *et al.* 1997) documented atrial flutter with 1 to 1 conduction (treatment), and atrioventricular conduction through an aberrant pathway (prophylaxis). Asymptomatic sinus bradycardia has been reported but at similar rates (18–36%) to CQ and S/P, suggesting malaria resolution as the cause (Kofi Ekue *et al.* 1983; Ekue *et al.* 1987). First degree heart block, sinus arrhythmia, non specific T wave changes and prolonged QTc intervals have also been observed in MQ treatment studies but detailed data are lacking (Harinasuta *et al.* 1987; Laothavorn *et al.* 1992). One treatment study documented a positive correlation between plasma MQ concentrations and the QTcB but all QTcB intervals were within normal limits (Touze *et al.* 2002). MQ does not produce significant QTc interactions when given with quinine or artemether/lumefantrine and MQ concentrations did not correlate with the QTc intervals (Supanaranond *et al.* 1997; Na-Bangchang *et al.* 1999; Bindschedler *et al.* 2000). However, the risk of QTc prolongation increased when MQ treatment failures were treated with halofantrine (Nosten *et al.* 1993). MQ preferentially inhibits the I_{Ks} channel, which may explain the synergistic QTc prolonging effect of MQ and halofantrine (Kang *et al.* 2001). There are few data on the effect of AS on the ECG intervals, one key study of intravenous (IV) AS in 21 severe malaria patients found no effect on the PR, QRS, QTcB and JTcB intervals (Maude *et al.* 2009).

Because many factors affect the QTc interval, obtaining pharmacokinetic (PK) data is crucial for interpreting QTc data. We report the effects on the ECG intervals in *Plasmodium falciparum* infected adults treated with AS and MQ.

Methods

Conduct of clinical trial

This randomised, safety and PK study of adults (weight >39 kg, no study drug allergies) with acute uncomplicated *P. falciparum* compared a new, fixed dose combination of AS and MQ to non fixed AS + MQ, the current standard in Thailand. A sample size of 25 patients per drug arm was deemed adequate for intense PK sampling. The study was conducted at the Hospital for Tropical Diseases, Bangkok, Thailand, from December 2004 to July 2005. It was approved by the ethical committees of the Faculty of Tropical Medicine, Mahidol University, and of the WHO. The trial is registered (http://www.controlled-trials.com/mrct/trial/228997/DNDi).

Drugs were administered as follows: (i) two fixed dose, AS/MQ (Farmanguihos, Brazil) tablets (one tablet = 100 mg AS, 200 mg MQ), daily for 3 days, (ii) AS [Arsumax® (50 mg of AS) Sanofi-Aventis, France] 4 mg/kg (D0), AS4 mg/kg + MQ15 mg/kg (D1), AS4 mg/kg + MQ10 mg/kg (D2); MQ = 250 mg MQ base (Roche, Basel, Switzerland). Patients were hospitalised for 28 days. Vital signs were monitored 4–6 hourly up until D21; thereafter daily. PK samples were taken at baseline, 0.25 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h and D1, 2, 3, 7, 14, 21, 28. The plasma was stored in cryotubes at −70 °C and sent to the Universiti Sains Malaysia for PK analysis (data to be presented elsewhere).

ECG methods

Standard, 12-lead ECGs (50 m/s, voltage sensitivity = 1 mV/cm) were done after patients lay rested for about 5 min at baseline, D3, 7 and 28 (if D7 ECG was abnormal). Day 3 (72 h) approximates the time of maximum MQ concentrations of the standard AS+MQ regimen (Price *et al.* 1999) and of the fixed dose AS/MQ used in this study (Krudsood *et al.* 2010) but was some 12 h after the mean Tmax when MQ was given as 8 mg/kg/day × 3 days (Ashley *et al.* 2006). ECGs were scanned, digitised and converted into an ECG Scan Extensible Mark-up Language (XML) format so ECG intervals (Lead II) could

be measured with on screen digital callipers. The first deflection of the QRS complex and the intersection of the descending part of the T wave (positive T wave) with the isoelectric line defined the start and end of the QT interval, respectively. If the U wave interrupted the T wave before it returned to baseline, the QT interval was measured as the nadir between T and U waves. If it was not clear whether a second deflection of the T wave was a U wave, it was included in the QT interval. All ECGs were reviewed by a cardiologist and all abnormal ECGs were reviewed again by a senior cardiologist.

The QTc intervals were calculated using three formulae: (i) Bazett's – QTcB = $QT/(RR)^{½}$, (ii) Fridericia – QTcF = $QT/(RR)^{0.33}$, and (iii) the new malaria formula (Price *et al.* 1998), QTcn = $QT/(RR)^{0.4}$. The JTc was derived from the QTc QRS difference, using an upper limit of normal (ULN) of 110 ms for the QRS interval. Because the Fridericia formula gave the best fit (regression line) between all the QT and RR intervals, the QTcF and JTcF were chosen as the primary intervals. All analyses were two sided and a P value of ≤ 0.05 was considered significant. No adjustment was made for multiple comparisons.

Statistical methods

Continuous data were analysed using the paired/unpaired t tests (normal data) or sign rank/Mann–Whitney U-tests (skewed data), as appropriate.

Categorical analyses of outlying QTc values (following ICH guidelines) were performed to ascertain the proportions of patients who had: (i) absolute QTcF interval increases ≥ 30 ms and ≥ 60 ms over baseline, (ii) QTcF increase >25% compared to baseline [33], (iii) QTcF interval ≥ 500 ms (iv) females with at least one QTcF ≥ 450 ms, and (v) males with at least one QTcF ≥ 430 ms.

A linear mixed effects model explored over time (D0, D3 and D7) the relationships between the QTcF and JTcF intervals and age, sex, treatment, temperature, MQ concentrations and HR. The model initially estimated times of measurements alone (i.e. no independent variables were used) to test for a linear trend in time and a random intercept for an initial effect (i.e. an effect without the influence of the independent variables). The random slope in time was then further tested for an increase over time of the QTcF and JTcF intervals. The random slope allows the calculation of an overall slope (coefficient) for the parameter being tested and the difference from this mean to be calculated for each subject. The times of measurements were excluded in the models when temperature, HR and MQ concentrations were assessed because time is already accounted for in these repeated factors. The random slope was not included in these models because the estimated variance-covariance matrix specified for the random intercept and the random slope were not positive. Therefore, estimating the correlation between the random coefficients could not be performed.

The clinical and ECG data were managed and analysed by a clinical research organisation, following an analysis plan with supplementary ECG and ECG PK analyses (WT, MV). Data were entered in Clintrial v4.3 database (Phase Forward, Waltham, Massachusetts, USA) and analysed with SAS® version 8 (SAS Institute, Cary, NC, USA) and SAS® version 9.3.1 (2^{nd} analyses). Further analyses (simple correlations, ANOVA, ANCOVA and logistic regression) are presented in Appendix S1.

Results

Descriptive analyses

Twenty five patients per drug arm were enrolled (Table 1). Most patients were male. Two patients developed early treatment failure and were withdrawn from the study on rescue treatment. 145 ECGs were analysed (D0 = 50, D3 = 47, D7 = 48). Because the ECG interval and HR data were very similar between the fixed and non fixed arms (data not shown), it was decided *post hoc* to combine the data. Serum potassium data were available mostly for D0 only. The mean and range values were very similar between the fixed and loose groups: 3.6 (2.8–4.4) *vs.* 3.7 (2.7–6.7) mmol/l.

The mean PR intervals changed little over time (Table 2). There was a trend towards a higher median D3 QRS interval *vs.* baseline. The significant changes over time were

Table 1 Patient characteristics at presentation and the fever (F) and parasite (P) clearance times (CT) after treatment

Variable	AS/MQ fixed $N = 25$	AS+MQ loose $N = 25$
Mean age in years (range)	26.6 (17–50)	28.9 (16–45)
Mean weight in kg (range)	50.1 (6.26)	51.0 (6.36)
Male, N (%)	19 (76)	22 (88)
Temperature (°C)*	38.5 (37.3–40)	38.2 (37–40)
Asexual parasitaemia (/μl)*	30 816 (69–170 800)	28 231 (20–140 280)
FCT (days)*	2.3 (1–7)†	2.3 (1–3)
PCT (days)*	1.89 (1–3.5)†	1.66 (0.5–3) days
T_{max} (h)‡	72 (19.1)	70.9 (13.5)
MQ C_{max} (ng/ml)‡	3279 (1252)	3239 (734)

*Mean (range).
†Based on 23 patients.
‡Mean (standard deviation).

Table 2 Summary of the ECG intervals, the temperature and heart rate data in patients who were treated with either the fixed or loose dose combinations of artesunate and mefloquine. Data are shown as mean (ranges) and compared by the paired *t*-test, except where indicated. Summary data are for all subjects on that day (D0 = 50, D3 = 47, D7 = 48); differences between Days are based on paired comparisons

	Day 0	Day 3	Day 7	D3 - D0	*P*	D7–D0	*P*
Temperature (^{0}C)	38.4 (37–40)	37.1 (36.5–37.7)	37.1 (36.5–39)	−1.2 (−3 to 0.5)	<0.000	−1.2 (−3 to 1.3)	<0.000
Heart rate/min	83 (59–112)	67 (44–84)	73 (59–101)	−15 (−43 to 20)	<0.000	−9 (−40 to 28)	0.0003
PR (ms)	148 (122–232)	148 (111–190)	145 (101–182)	0 (−61 to 36)	0.97	−3 (−99 to 30)	0.23
QRS* (ms)	91 (75–118)	94 (63–135)	91 (73–111)	3 (−18 to 47)	0.055	1 (−26 to 16)	0.67
QT (ms)	352 (290–429)	394 (301–501)	375 (310–425)	42 (−55 to 128)	<0.000	23 (−70 to 132)	0.0001
JT (ms)	260 (194–327)	299 (219–393)	282 (218–329)	39 (−41 to 122)	<0.000	22 (−61 to 135)	0.0001
QTcF (ms)	389 (344–434)	407 (319–504)	399 (357–443)	18 (−29 to 98)	<0.000	11 (−33 to 92)	0.0027
JTcF (ms)	288 (231–344)	309 (231–395)	301 (251–342)	21 (−31 to 97)	<0.000	13 (−27 to 110)	0.0006
QTcn (ms)	397 (352–438)	410 (323–504)	404 (362–455)	13 (−36 to 97)	0.0009	8 (−33 to 83)	0.01
JTcn (ms)	294 (239–438)	311 (235–395)	305 (258–344)	18 (−39 to 36)	0.0001	11 (−23 to 104)	0.0015
QTcB (ms)	409 (362–458)	414 (329–505)	412 (366–473)	5 (−49 to 96)	0.18	3 (−32 to 68)	0.28
JTcB (ms)	303 (252–354)	314 (239–396)	311 (269–357)	12 (−51 to 96)	0.004	8 (−25 to 94)	0.01

*Median (range), QRS was not normally distributed. QRS comparisons used the Sign test.

in the mean HR, the mean QT/QTc and JT/JTc values (Table 2 and Figure 1). Seven of 47 (14.9%) patients developed sinus bradycardia (<60/min) on their D3 ECGS, which was still present in 2 of 48 (4.16%) patients on the D7 ECG. Pulse rate ranges for the latter two patients were: (i) 60–76, 66–78 on D3, (ii) 68–80, 76–80 on D7, (iii) 66–80, 76–84 on D8, and (iv) 66–84, 72–84 on D9. Mean D3 mefloquine concentrations ([MQ])s were similar ($P = 0.42$) between patients with (2789 ng/ml) and without (3156 ng/ml) sinus bradycardia. No patients had sinus arrhythmia. Two patients developed transient non specific T wave changes.

No MQ was detected on D0. [MQ]s on D3 and D7 ranged (mean) from 1346 to 5796 (3095) and 600 to 2917 (1721) ng/ml, respectively, and were similar between the fixed and non fixed arms on D3 (3013 *vs.* 3165 ng/ml, $P = 0.65$) and D7 (1577 *vs.* 1846 ng/ml, $P = 0.16$).

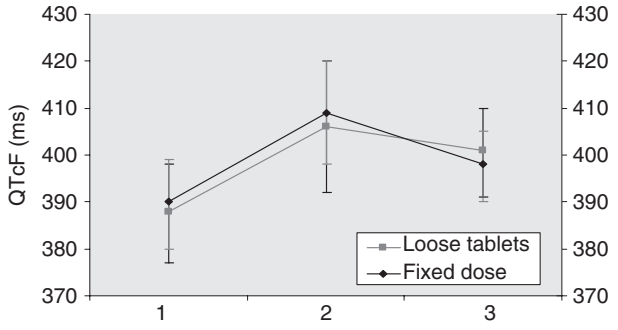

Figure 1 Mean (95% CIs) QTcF intervals of the first (1), second (2) and third ECGs for the fixed and loose combinations of artesunate and mefloquine in *P. falciparum* infected patients.

Categorical analyses of outlying QTcF values

In total, 10 fixed and 11 non fixed patients had 33 ICH defined alert values, including 3 QTcF values at baseline. Fourteen and 7 patients had increases in the QTcF ≥ 30 to <60 ms on D3 and D7, respectively, and 3 (D3 = 2) patients had QTcF increases ≥ 60 ms. Eleven of the 14 and 4 of the 7 patients had QTcF values within the normal range for their sex (Table 3). One male (loose arm) with a normal baseline QTcF 406 ms developed a D3 QTcF of 504 ms that declined to 433 ms by D7; corresponding changes in heart rates were small: 63, 61, 70 beats/min; his D3 and D7 [MQ]s were 2458.72 and 1820.29 ng/ml. Another male (fixed arm) patient had a QTcF increase of 24.4 and 26.6% over baseline: 346, 430, 438 ms with large declines in heart rates: 102, 65, 67 beats/min.

Linear mixed effects model

The means of the random intercept estimates (i.e. the effect of QTcF or JTcF without the influence of any factors/independent variables) were 393.42 ms for the QTcF and 292.69 ms for the JTcF (Table 4). The increase in time (random slope) was 0.06401 ms per hour (QTcF) for an increase of 4.61 ms at D3 (72 h) and 10.75 ms at D7; for the JTcF, the increase was 0.07945 ms/h: 5.72 ms (D3) and 13.35 ms (D7). No significant effects were seen for the covariates drug formulation, age and sex.

Significant effects were observed in the modelling of QTcF and JTcF with [MQ], but not the MQ AUC values, temperature and HR. Increasing blood MQ concentrations at each time of measurement led to a mean increase of 17.96 ms (QTcF) and 19.52 ms (JTcF) at D3, and 9.40 and

Table 3 Patients with ICH alert values* of the absolute QTcF intervals and their changes from baseline

		Day 0	Day 3	Δ D3–0	Δ D3–0%	Day 7	Δ D7–0	Δ D7–0%	HR D0	HR D3	HR D7	D3 [MQ]	D7 [MQ]
1	Female	387	417	30	7.8	387	0	0.0	90	68	72	2542	1698
2	Male	396	444	48	12.1	412	16	4.0	91	67	71	NA	NA
3	Male	378	422	44	11.6	403	25	6.6	91	76	63	2181	1742
4	Male	344	386	42	12.2	365	21	6.1	84	66	79	2611	1160
5	Male	434	405	−29	−6.7	413	−21	−4.8	71	60	64	3325	2174
6	Male	406	504	98	24.1	433	27	6.7	63	61	70	2458	1820
7	Male	377	415	38	10.1	398	21	5.6	90	58	68	2594	1127
8	Male	382	418	36	9.4	392	10	2.6	95	68	69	3045	1849
9	Male	373	NA	NA	NA	431	58	15.5	93	NA	80	3089	1937
10	Male	347	319	−28	−8.1	378	31	8.9	100	72	73	4652	2917
11	Male	357	366	9	2.5	393	36	10.1	94	55	64	2300	1275
12	Male	392	423	31	7.9	417	25	6.4	84	76	79	3199	1997
13	Male	432	419	−13	−3.0	409	−23	−5.3	61	62	89	3786	2210
14	Male	371	427	56	15.1	399	28	7.5	74	44	62	1419	600
15	Male	413	457	44	10.7	443	30	7.3	112	69	89	3178	1421
16	Male	379	411	32	8.4	388	9	2.4	93	68	76	1345	1252
17	Male	357	414	57	16.0	395	38	10.6	86	79	73	3159	1748
18	Male	409	436	27	6.6	389	−20	−4.9	97	71	101	1748	1484
19	Male	432	441	9	2.1	420	−12	−2.8	70	65	67	3269	1136
20	Male	381	416	35	9.2	423	42	11.0	89	53	59	3535	791
21	Male	346	430	84	24.3	438	92	26.6	102	65	67	3225	2371

*ICH alert values are:
(i) post baseline QTcF increases ≥ 30–<60 ms and ≥ 60 ms.
(ii) post baseline QTcF values of ≥ 430–<450 ms & ≥ 450 ms (males).
(iii) post baseline QTcF values of ≥ 450–<470 ms & ≥ 470 ms (females).
(iv) post baseline QTcF values ≥ 500 ms.

10.21 ms at D7, respectively. An increase in [MQ] of 1 ng/ml lead to an increase of 0.006 ms in the QTcF as well as the JTcF intervals.

A mixed model was run to estimate the mean changes in [MQ] between D0-D3 and D0-D7; the mean differences were 856 ng/ml and 642 ng/ml, respectively. Allowing for the modelling of 2 slopes for the same periods, the model found a mean increase of 0.34 in QTcF and 0.37 ms in JTcF, respectively, between D0 and D3, and a mean decrease of 0.08 ms in QTcF and JTcF between D3 and D7.

By contrast, increasing values of temperature and HR decreased the QTcF and JTcF values (Table 4). An increase in temperature of 1 °C led to a decrease in QTcF of 7.99 ms and JTcF of 10.25 ms. An increase of 1 beat/min in the HR led to a decrease of 0.8 ms in QTcF and 1.1 ms in JTcF.

Using a manual descending, model building approach of all fixed (treatment arm, sex and age) and random (MQ concentrations, temperature and HR) variables, the HR was the only significant variable ($P = 0.001$) over time in the model that best explained the changes in the QTcF and JTcF intervals.

Discussion

This study has shown that the mean QTcF and JTcF intervals rose initially then fell during treatment of drug resistant *P. falciparum*. Our analyses found consistently that these observations were related significantly and inversely to changes in the HR which slowed as malaria resolved.

Our data have implications for other antimalarial drugs because disease resolution will always explain part of the increase in the QTc interval and, possibly, the further slowing of the HR because ECGs are done after a short resting period. However, there are three caveats for practising clinicians. It is important to use therapeutic doses, be wary of co-prescribing AS/MQ with drugs that could prolong the QTc interval (e.g. antiemetics, AS/MQ should *never* be used for treating halofantrine failures), and to not disregard cardiac symptoms. There is a move towards age based dosing of antimalarial drugs in some malaria endemic countries; therefore, some patients will receive doses above the therapeutic range which could adversely affect tolerability (Taylor *et al.* 2006).

Table 4 Random effects model examining the effects of heart rate, mefloquine concentration and temperature on the QTcF and JTcF intervals

Effect	Estimate	Standard error	df	*t* value	$P > \|t\|$
QTcF = f([t])					
Intercept	393.31	3.6865	46	106.69	<.0001
TIME_in_hrs	0.06401	0.02354	42	2.72	0.0095
QTcF = f([MQ])					
Intercept	389.89	2.7191	42	143.39	<.0001
MQ_conc	0.005958	0.0018	42	3.31	0.0019
QTcF = f([T°])					
Intercept	699.13	75.323	49	9.28	<.0001
VSTEMP	−7.9988	2.0026	47	−3.99	0.0002
QTcF = f([HR])					
Intercept	457.49	10.1013	49	45.29	<.0001
ECGHR	−0.7891	0.1289	47	−6.12	<.0001
JTcF = f([t])					
Intercept	292.6	3.5372	46	82.72	<.0001
TIME_in_hrs	0.07945	0.02668	42	2.98	0.0048
JTcF = f([MQ])					
Intercept	289.71	3.3848	42	85.59	<.0001
MQ_conc	0.006476	0.001569	42	4.13	0.0002
JTcF = f([T°])					
Intercept	684.76	84.0703	49	8.15	<.0001
VSTEMP	−10.2509	2.2351	47	−4.59	<.0001
JTcF = f([HR])					
Intercept	379.98	10.3313	49	36.78	<.0001
ECGHR	−1.0769	0.1322	47	−8.14	<.0001

There are few PK ECG data on MQ in patients. Touze *et al.* (2002) found no significant changes in the QTcB interval or QTcB dispersion in 15 MQ treated, falciparum infected patients but, by simple regression, plasma MQ concentrations correlated positively with both variables. Our model found also a positive relationship between the QTcF and JTcF and MQ concentration with a mean increase of 0.34 in QTcF and 0.37 ms in JTcF by D3 followed by a mean decrease by D7 of 0.08 ms for both the QTcF and JTcF. This relationship disappeared when all factors were included in the model to adjust for their effects on each other and the HR remained the only significant factor for the change in the QTcF and JTcF. These findings suggest a neutral effect of MQ concentrations arising from therapeutic dosing on the QTcF and JTcF intervals but do not exclude the possibility of a weak positive relationship.

A low (approximately 15%) proportion of our patients developed new sinus bradycardia on D3 that was independent of D3 MQ concentrations and resolved mostly by D7, similar to the findings of others (Ekue *et al.* 1987). Interestingly, the pulse rates of the two ECG bradycardic patients did not show bradycardia, suggesting the short rest before the ECG was an important contributory factor. Malaria itself may have a small effect on the ECG intervals. von Seidlein *et al.* (1997) found a weak positive correlation between the D0 parasitaemia and the QTcB interval in falciparum infected children, whereas we found no correlations between D0 parasitaemia and temperature and PR, QRS, QTcF and JTcF intervals. More data are needed to assess the possible malaria effects on the ECG.

The ICH guidelines recommend the Bazett and Fridericia correction formulae for ECG studies and focus on healthy volunteer studies in Western populations where ischaemic heart disease is prevalent. Using either formula in placebo controlled studies, a net mean increase in QTc interval of 'around' 5 ms (upper 95% CI of 10 ms) is a 'concerning level' and an absolute increase to >500 ms is of 'particular concern.' We chose the Fridericia correction formula based on the best fit of all of the QT HR data and explored the JT and JTc intervals which are not in the ICH guidelines.

One patient had a D3 QTcF exceeding 500 ms without a substantial fall in his HR. His D3 [MQ] was below the sample mean so this increase might be explained partly by MQ acting on 'sensitive' I_{Ks} channels. Another patient had a percentage increase over baseline exceeding the 25% threshold used by some malariologists (Nosten *et al.* 1993) but he had marked falls in his HR. Several patients had QTcF increases of ≥30 and ≥60 ms over baseline but most had QTcF values in the normal range. The mean D3 D0 change in the QTcF was 18 ms (upper 95% CI of 26 ms) which was higher than the QTcn values of 13 and 21 ms, respectively. Of note is that the least accurate correction formula, Bazett, had the least mean QTcB increase of 5 ms with an upper 95% CI of 12 ms. These differing mean changes highlight the variation in results obtained by using different formulae and leave the question open as to which formula is optimal. The mean QTcF and QTcn increases over baseline were higher than the concerning values of the ICH guidelines and suggests that the ICH limits may not be applicable to febrile patients. We also explored the use of the JTc interval and defined a clinically significant value as any value exceeding the ULN because an increase in ventricular repolarisation is important for developing TdP. However, there are no regulatory guidelines defining the JTc limits of concern. More research is needed to define these and to compare the usefulness of the JTc *vs.* the QTc intervals.

Our study had limitations. The sample size was small and recruited predominantly fit young men. This limits the applicability of our findings to populations where ischaemic heart disease is rare. Only three ECGs per patient were performed. Performing several ECGs at each time point to reduce the intra individual variation would have been more informative (http://www.diahome.org/en/Resources/Publications/JournalsandMagazines.htm, accessed April 2010). We could not perform 24 h Holter monitoring

which would have yielded more data. We did not measure the red cell transketolase (for thiamine deficiency), serum calcium and magnesium and only measured the serum potassium at baseline. There were multiple analyses and some statistically significant results may have occurred by chance.

To conclude, the mean QTc interval increase was consistently and independently related to the fall in HR. Although the mixed effects model suggested a possible weak positive relationship with MQ concentrations, this is of limited clinical significance at therapeutic doses.

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References

Ashley EA, Stepniewska K, Lindegardh N *et al.* (2006) Population pharmacokinetic assessment of a new regimen of mefloquine used in combination treatment of uncomplicated falciparum malaria. *Antimicrobial Agents and Chemotheraphy* **50**, 2281–2285.

Batchvarov V & Malik M (2000) Measurement and interpretation of QT dispersion. *Progress in Cardiovascular Diseases* **42**, 325–344.

Bindschedler M, Lefevre G, Ezzet F, Schaeffer N, Meyer I & Thomsen MS (2000) Cardiac effects of co-artemether (artemether/lumefantrine) and mefloquine given alone or in combination to healthy volunteers. *European Journal of Clinical Pharmacology* **56**, 375–381.

Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED & Keating MT (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* **80**, 795–803.

Dessertenne F (1966) [Ventricular tachycardia with 2 variable opposing foci]. *Archives des Maladies du Coeur et des Vaisseaux* **59**, 263–272.

Ekue JM, Phiri DE, Sheth UK & Mukunyandela M (1987) A double-blind trial of a fixed combination of mefloquine plus sulfadoxine-pyrimethamine compared with sulfadoxine-pyrimethamine alone in symptomatic falciparum malaria. *Bulletin of the World Health Organisation* **65**, 369–373.

Fonteyne W, Bauwens A & Jordaens L (1996) Atrial flutter with 1:1 conduction after administration of the antimalarial drug mefloquine. *Clinical Cardiology* **19**, 967–968.

Gundersen SG, Rostrup M, von der Lippe E, Platou ES, Myrvang B & Edwards G (1997) Halofantrine-associated ventricular fibrillation in a young woman with no predisposing QTc prolongation. *Scandinavian Journal of Infectious Diseases* **29**, 207–208.

Gupta RK, Van Vugt M, Paiphun L *et al.* (2005) Short report: no evidence of cardiotoxicity of atovaquone-proguanil alone or in combination with artesunate. *American Journal of Tropical Medicine and Hygiene* **73**, 267–268.

Harinasuta T, Bunnag D, Vanijanond S *et al.* (1987) Mefloquine, sulfadoxine, and pyrimethamine in the treatment of symptomatic falciparum malaria: a double-blind trial for determining the most effective dose. *Bulletin of the World Health Organisation* **65**, 363–367.

Jaspers CA, Hopperus Buma AP, van Thiel PP, van Hulst RA & Kager PA (1996) Tolerance of mefloquine chemoprophylaxis in Dutch military personnel. *American Journal of Tropical Medicine and Hygiene* **55**, 230–234.

Kang J, Chen XL, Wang L & Rampe D (2001) Interactions of the antimalarial drug mefloquine with the human cardiac potassium channels KvLQT1/minK and HERG. *Journal of Pharmacology and Experimental Therapeutics* **299**, 290–296.

Kofi Ekue JM, Ulrich AM, Rwabwogo-Atenyi J & Sheth UK (1983) A double-blind comparative clinical trial of mefloquine and chloroquine in symptomatic falciparum malaria. *Bulletin of the World Health Organisation* **61**, 713–718.

Krudsood S, Looareesuwan S, Tangpukdee N *et al.* (2010) New fixed-dose artesunate-mefloquine formulation against multidrug-resistant Plasmodium falciparum in adults: a comparative phase IIb safety and pharmacokinetic study with standard-dose nonfixed artesunate plus mefloquine. *Antimicrobial Agents and Chemotherapy* **54**, 3730–3737.

ter Kuile FO, Nosten F, Luxemburger C *et al.* (1995) Mefloquine treatment of acute falciparum malaria: a prospective study of non-serious adverse effects in 3673 patients. *Bulletin of the World Health Organisation* **73**, 631–642.

Laothavorn P, Karbwang J, Na Bangchang K, Bunnag D & Harinasuta T (1992) Effect of mefloquine on electrocardiographic changes in uncomplicated falciparum malaria patients. *Southeast Asian Journal of Tropical Medicine and Public Health* **23**, 51–54.

Malvy D, Receveur MC, Ozon P *et al.* (2000) Fatal cardiac incident after use of halofantrine. *Journal of Travel Medicine* **7**, 215–216.

Maude RJ, Plewes K, Faiz MA *et al.* (2009) Does artesunate prolong the electrocardiograph QT interval in patients with severe malaria? *American Journal of Tropical Medicine and Hygiene* **80**, 126–132.

Mbai M, Rajamani S, January CT *et al.* (2002) The anti-malarial drug halofantrine and its metabolite N-desbutylhalofantrine block HERG potassium channels. *Cardiovascular Research* **55**, 799–805.

Monlun E, Le Metayer P, Szwandt S *et al.* (1995) Cardiac complications of halofantrine: a prospective study of 20 patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 430–433.

Mytton OT, Ashley EA, Peto L *et al.* (2007) Electrocardiographic safety evaluation of dihydroartemisinin piperaquine in the treatment of uncomplicated falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **77**, 447–450.

Na-Bangchang K, Tan-Ariya P, Thanavibul A, Riengchainam S, Shrestha SB & Karbwang J (1999) Pharmacokinetic and pharmacodynamic interactions of mefloquine and quinine. *International Journal of Clinical Pharmacology Research* **19**, 73–82.

Ngouesse B, Basco LK, Ringwald P, Keundjian A & Blackett KN (2001) Cardiac effects of amodiaquine and sulfadoxine-pyrimethamine in malaria-infected African patients. *American Journal of Tropical Medicine and Hygiene* **65**, 711–716.

Nosten F, ter Kuile FO, Luxemburger C *et al.* (1993) Cardiac effects of antimalarial treatment with halofantrine. *Lancet* **341**, 1054–1056.

Price RN, Nosten F & White NJ (1998) Prolongation of the QTc interval in African children treated for falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **59**, 503.

Price R, Simpson JA & Teja-Isavatharm P (1999) Pharmacokinetics of mefloquine combined with artesunate in children with acute falciparum malaria. *Antimicrobial Agents and Chemotheraphy* **43**, 341–346.

Ribeiro IR & Olliaro P (1998) Safety of artemisinin and its derivatives. A review of published and unpublished clinical trials. *Medecine Tropicale (Mars)* **58**(3 Suppl), 50–53.

Richter J, Burbach G, Hellgren U, Dengler A & Bienzle U (1997) Aberrant atrioventricular conduction triggered by antimalarial prophylaxis with mefloquine. *Lancet* **349**, 101–102.

Sanguinetti MC, Jiang C, Curran ME & Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. *Cell* **81**, 299–307.

vn Seidlein L, Jaffar S & Greenwood B (1997) Prolongation of the QTc interval in African children treated for falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **56**, 494–497.

Supanaranond W, Suputtamongkol Y, Davis TM *et al.* (1997) Lack of a significant adverse cardiovascular effect of combined quinine and mefloquine therapy for uncomplicated malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 694–696.

Taylor WR, Terlouw DJ, Olliaro PL, White NJ, Brasseur P & ter Kuile FO (2006) Use of weight-for-age-data to optimize tablet strength and dosing regimens for a new fixed-dose artesunate-amodiaquine combination for treating falciparum malaria. *Bulletin of the World Health OrganisationBulletin of the World Health Organisation* **84**, 956–964.

Touze JE, Bernard J, Keundjian A *et al.* (1996) Electrocardiographic changes and halofantrine plasma level during acute falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **54**, 225–228.

Touze JE, Heno P, Fourcade L *et al.* (2002) The effects of antimalarial drugs on ventricular repolarization. *American Journal of Tropical Medicine and Hygiene* **67**, 54–60.

White NJ (2007) Cardiotoxicity of antimalarial drugs. *Lancet Infectious Diseases* **7**, 549–558.

Yap YG & Camm AJ (2003) Drug induced QT prolongation and torsades de pointes. *Heart* **89**, 1363–1372.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Correlations

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