Pharmacokinetics and electrocardiographic pharmacodynamics of artemether-lumefantrine (Riamet®) with concomitant administration of ketoconazole in healthy subjects

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Aims To evaluate whether the potent CYP3A4 inhibitor ketoconazole has any influence on the pharmacokinetic and electrocardiographic parameters of the antimalarial co-artemether (artemether-lumefantrine) in healthy subjects.

Methods Sixteen subjects were randomized in an open-label, two period crossover design study. Subjects received a single dose of co-artemether (day 1) either alone or in combination with multiple oral doses of ketoconazole (400 mg on day 1 followed by 200 mg o.d. for 4 additional days). Serial blood samples were taken and assayed for artemether and its main active metabolite dihydroartemisinin (DHA), and lumefantrine.

Results The pharmacokinetics of artemether, its metabolite DHA, and lumefantrine were influenced by the presence of ketoconazole. $AUC(0,\infty)$ was increased from 320 to 740 ng ml^{-1} h (ratio 2.4, 90% CI 2.00, 2.86) for artemether, from 331 to 501 ng ml^{-1} h (ratio 1.7, 90% CI 1.40, 1.98) for DHA, and from 207 to 333 μg ml^{-1} h (ratio 1.7, 90% CI 1.23, 2.21) for lumefantrine in the presence of ketoconazole. C_{max} also increased in similar proportions for the three compounds (ratio 2.2 (90% CI 1.78, 2.83), 1.4 (90% CI 1.12, 1.74), and 1.3 (90% CI 0.96, 1.64), respectively). The terminal elimination half-life was increased for artemether (2.5 *vs* 1.9 h, 90% CI 1.12, 1.72) and DHA (3.1 *vs* 2.1 h, 90% CI 0.02, 3.36), but remained unchanged for lumefantrine (88 *vs* 95 h, 90% CI 0.81, 1.04). These increases in exposure to the antimalarial combination were much smaller than observed with food intake (up to 16 fold), and were not associated with increased side-effects or changes in electrocardiographic parameters. The study medications were well tolerated.

Conclusions The concurrent administration of ketoconazole with co-artemether led to modest increases in artemether, DHA, and lumefantrine exposure in healthy subjects. Dose adjustment of co-artemether is probably unnecessary in falciparum malaria patients when administered in association with ketoconazole or other potent CYP3A4 inhibitors.

Keywords: artemether, ECG, ketoconazole, lumefantrine, malaria, QTc

Introduction

Malaria is a leading cause of mortality and morbidity in developing areas of the world, and remains a major health problem in endemic regions. Resistance to available drugs is increasing, thus creating a clear need for new drugs that are well tolerated and simple to use.

Co-artemether is an oral fixed-dose combination tablet of artemether (a derivative of artemisinin) and lumefan-

trine, an antimalarial synthesized and developed by the Academy of Military Medical Sciences in Beijing, People's Republic of China. This combination was registered in China in 1992 for the treatment of *Plasmodium falciparum* malaria, and has been developed further by Novartis Pharmaceuticals (Coartem®/Riamet®). To date, Coartem®/Riamet® (Exafal® in Pakistan) has been approved in more than 60 countries, mainly in Africa, Asia, South America, and Europe.

Artemether is characterized by a rapid onset of schizonticidal action, but has a short elimination half-life (2–3 h). However, recrudescence is frequent when artemether is employed as monotherapy [1], unless high dosages are given over several days [2–4]. By contrast,

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lumefantrine has a longer elimination half-life of several days [5, 6] and is associated with a low recrudescence rate [7, 8], but has a slower onset of action. The rationale for the drug combination is to combine the benefits of the rapid onset of action of artemether with the long duration of action and high cure rate of lumefantrine in a single oral formulation. The combination represents a short, simple to administer, and highly effective antimalarial treatment, and is also likely to improve compliance [8]. This combination approach of an artemisinin derivative with a long-acting other antimalarial is now recommended by the most recent guidelines (World Health Organization [WHO] Model List of Essential Drugs, 1999), since it protects more effectively against the development of resistance [9–14]. The therapeutic dosage regimen in adults consists of repeated administration over 2 days of four doses of co-artemether, comprising four consecutive doses of four tablets each (80 mg artemether +480 mg lumefantrine per dose). In areas of multiple drug-resistant malaria, such as Thailand, and for stand-by emergency treatment, an intensive 3 day course including two additional doses is recommended.

The pharmacokinetics of artemether, its active metabolite dihydroartemisinin (DHA), and lumefantrine have been characterized after oral doses of co-artemether in healthy subjects and in paediatric and adult patients with falciparum malaria [5, 6, 14–16]. *In vitro* and *in vivo* data indicated that artemether and lumefantrine are predominantly metabolized by cytochrome P450 3A4 (CYP3A4) [5, 17]. Therefore, concomitant intake of drugs that inhibit CYP3A4 activity could result in increased plasma concentrations of artemether and/or lumefantrine, potentially leading to unwanted pharmacodynamic effects.

Ketoconazole, an imidazole derivative and broad-spectrum antifungal agent, is among the most potent inhibitors of CYP3A4. It has been shown to impair the oxidative metabolism of a wide range of clinically used drugs such as antihistamines, immunosuppressives, benzodiazepines and HIV protease inhibitors [18–22]. Clinically significant interactions were reported between ketoconazole and terfenadine or astemizole or tolbutamide leading to QTc interval prolongation and torsade de pointes [23–27].

In light of these findings, the present study was conducted to investigate the effect of ketoconazole on the pharmacokinetics of artemether, its active main metabolite DHA, and lumefantrine. Although co-artemether, in contrast to other antimalarials like quinine or halofantrine, has never been reported to cause any relevant cardiotoxicity when given alone [28–31] or in combination with mefloquine [32] or quinine [16], electrocardiographic parameters were closely monitored during this study.

Methods

Subjects

Sixteen healthy Caucasian nonsmokers, male and female subjects aged 19–49 years (mean 29.7) and weighing 61.4–87.4 kg (mean 78.6) participated in the trial. The study excluded subjects with a history of drug sensitivity or allergy, heart disease or significant ECG abnormalities (e.g. QTc intervals >440 ms), psychiatric disturbances, a recent history of alcohol abuse, and subjects who required regular medication. Subjects were excluded if they had taken any prescription medication within 4 weeks, or over-the-counter medications within 2 weeks of the start of the study. Furthermore, only females surgically sterilized prior to study entry were admitted. The trial was conducted at MDS Pharma Services (MDSPS), Belfast, Northern Ireland, in accordance with the World Medical Association's Declaration of Helsinki, and Good Clinical Practice. Ethics Committee approval of the protocol, consent form, and volunteer information document was obtained from the Queen's University Independent Review Board. All subjects provided written informed consent before participating in the trial.

Dosage forms and doses

Co-artemether (Riamet®, Novartis Pharma AG, Basel, Switzerland): tablets, each containing 20 mg artemether and 120 mg lumefantrine. Co-artemether was administered on day 1 as a single dose corresponding to four tablets (total dose: 80 mg artemether/480 mg lumefantrine).

Ketoconazole (Nizoral®, Janssen-Cilag, Saunderton, High Wycombe, United Kingdom) was administered on day 1 (400 mg, 2 tablets), followed by 200 mg (1 tablet) once daily on days 2–5.

Study design and rationale

Either a single dose of artemether-lumefantrine (80/ 480 mg), or five subsequent once-daily doses of ketoconazole (400 mg on day 1 and 200 mg on days 2–5) in combination with co-artemether (80/480 mg) on day 1 were administered in a randomized, non-blind, two period crossover design, with a 7 week washout ($t_{1/2}$ of lumefantrine is 3–6 days). Each subject participated in a screening period (day −21 to day −2), a baseline period (12 h prior to dosing), and the two treatment periods. Subjects were confined to the study site until after the 48 h PK and safety assessments. They returned to the study centre on days 3, 4 and 5 to receive their daily dose of ketoconazole and/or for blood sampling and safety assessments. Since food is known to increase the

systemic exposure to both ketoconazole [33] and artemether-lumefantrine [5, 34], the study medications were taken with a high fat breakfast (2 eggs fried, 2 strips of bacon, 1 slice of toast with butter, 2 hash brown potatoes, 240 ml of full-fat milk). Fluids that did not contain caffeine or grapefruit juice were allowed *ad libitum*.

Pharmacokinetic analysis

Blood samples were taken for the analysis of artemether and its active metabolite DHA in plasma at predose (0 h), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, and 48 h post dose of co-artemether. Blood samples were taken for the analysis of lumefantrine in plasma at predose (0 h), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 168, 216, and 264 h postdose of co-artemether. Blood was collected in lithium-heparinized tubes, and centrifuged without delay at 4 °C at 2200 g for 5 min. Plasma was then split into three aliquots that were kept in polypropylene screw-cap tubes at −70 °C until analysis. Artemether and DHA were measured by high-performance liquid chromatography (h.p.l.c.) − mass spectrometry [35]. Lumefantrine was measured by h.p.l.c. with ultraviolet detection [36]. The within-assay accuracy data of control samples (in percent of nominal concentration) were in the range 97–102% for artemether, 95% for DHA, and 102–107% for lumefantrine, with coefficients of variation (CV) of 10–12%, 9–16%, and 6–8%, respectively. Limits of quantification were 5 ng ml^{-1} for artemether and DHA, and 50 ng ml^{-1} for lumefantrine.

The following model-independent pharmacokinetic parameters (WinNonlin Professional, Version 3.1, Pharsight Corporation, Mountain View, CA, USA) were determined from plasma concentration data: C_{max}, highest observed plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$, apparent terminal elimination half-life (calculated as $t_{1/2} = \ln 2/\lambda_z$, where λ_z is the rate constant associated with the terminal phase of elimination, estimated by log-linear least-squares regression through the last concentration points different from zero); AUC(0,last), area under the plasma concentration-time curve calculated by the trapezoidal (linear-log) method over the time interval 0 h to the last time point with a concentration different from zero (C_t). AUC(0,∞), AUC extrapolated to time infinity calculated as AUC(0,last) + C_t/λ_z. Concentrations below the limit of quantification of the relevant analytical method were taken as zero in descriptive statistics and pharmacokinetic calculations.

Safety assessments

Medical history, physical examination, vital signs, routine clinical laboratory tests, ECGs and urine screens for drugs

and alcohol were performed at screening and at specific time points during the trial (ECGs were recorded frequently throughout the trial period), then at the follow up visit. Adverse events were recorded continuously throughout the trial, and the onset, duration, severity, and relationship to the trial drugs were noted.

Standard 12-lead ECGs were recorded at screening, baseline (both treatment periods), immediately prior to dosing (time 0 h), and at 2, 8, 10, 12, 24, 36, and 48 h postdose of co-artemether. QTc-interval was calculated by using the Bazett formula QTc = QT/√RR [37, 38] to correct for the influence of heart rate. For subjects' safety, the investigator evaluated the PR-, QRS- and QT-intervals visually on an ongoing basis.

Statistical analysis

The power calculation for determination of sample size was based on detecting a two-fold difference in exposure (AUC). Based on a previous volunteer study [6], and assuming no drug–drug interaction, 13 subjects were required to demonstrate with 80% power that the 90% confidence interval (90% CI) for AUC$_{combination}$/ AUC$_{co-artemether}$ lies within the interval 0.5–2.0. Because of the long duration of the study, 16 subjects were enrolled in case of withdrawals.

To investigate the pharmacokinetic interaction effects between treatments, AUC(0,last), C_{max}, t_{max}, and $t_{1/2}$ of artemether, DHA, and lumefantrine were compared by analysis of variance (ANOVA). Values were transformed to the natural logarithm scale and then used as the dependent variable with treatment, sequence, subject within sequence, and period as factors. t_{max} was analysed by the Wilcoxon rank sum test. In accordance with recent FDA guidance for industry on *In Vivo* Drug Metabolism/Drug Interaction Studies (http://www.fda.gov/cder/guidance/ 2635fnl.pdf), comparison between treatment was based on 90% CIs. No *P* values were given according to this guidance.

The GLM procedure (SAS Release 8.1 for Open VMS, SAS Institute Inc.) was used in the above ANOVA evaluations. Estimated differences and their standard errors between pairs of treatments (or other main effects) were obtained using the ESTIMATE statement. These were exponentiated, i.e. antilogarithmically transformed, to obtain estimated ratios. Similarly, 90% CIs for the ratios were also obtained. The intersubject coefficient of variation of the dependent variable was obtained by taking the square root of the residual variance after fitting the GLM model. The GLM procedure was used for Wilcoxon test of t_{max}. Only descriptive statistics were done for cardiographic parameters (QTc).

Results

Safety and tolerability

Co-artemether given as a single dose or coadministered with multiple doses of ketoconazole appeared to be safe and well tolerated in this healthy male and female population.

There were no serious adverse events (AEs). There were 9 AEs in 5 (31%) of the 16 subjects, mostly of mild intensity. These 9 AEs included sunburn, increased GGT, increased blood CK, and dizziness. The only AE considered to be possibly treatment-related was the episode of dizziness that occurred 24 h following co-artemether alone treatment and lasted 1 min. Three subjects withdrew from the study, one due to a bloodshot eye, abrasion, contusion, and hand fracture subsequent to a bike accident (unrelated to the study drugs), one due to increased GGT (unrelated to the study drugs), and one due to withdrawal of consent. No clinically significant deviations in laboratory parameters (haematology, blood chemistry and urinalysis) were observed in any of the treatment groups.

ECG parameters (PR-interval, QRS-complex, heart rate, QT- and QTc-interval) remained well within normal limits in both treatment groups. Except for one single borderline peak QTc value of 465 ms (causally unrelated to the study medication) observed in a male 2 h after co-artemether alone treatment, no clinically significant modifications in QTc intervals were observed in any subject and treatment, and no abnormal clinical signs or symptoms were reported. No clinically significant trends in ventricular rate, RR- or PR-interval were observed for any subject.

Pharmacokinetics

Artemether–Dihydroartemisinin

The mean plasma profiles of artemether and dihydroartemisinin are shown in Figure 1, and the pharmacokinetic parameters and statistical results are summarized in Table 1. Co-administration of co-artemether with ketoconazole was associated with an increase in overall artemether and DHA exposure compared to co-artemether alone. $AUC(0,\infty)$ increased by 2.4 fold (from 320 to 740 ng ml^{-1} h) for artemether, and 1.7 fold (from 331 to 501 ng ml^{-1} h) for DHA. Similarly, C_{max} increased by 2.2 fold (from 104 to 225 ng ml^{-1}) for artemether, and 1.4 fold (from 104 to 142 ng ml^{-1}) for DHA. These changes were associated with a slight increase in $t_{1/2}$ for both artemether (1.9 *vs* 2.5 h) and DHA (2.1 *vs* 3.1 h). There was no treatment difference in t_{max} for both artemether and DHA. Since the 90% CIs for ratios of C_{max} and of AUC means both fell outside the no effect 0.80–1.25 range, the effect of ketoconazole was statistically significant.
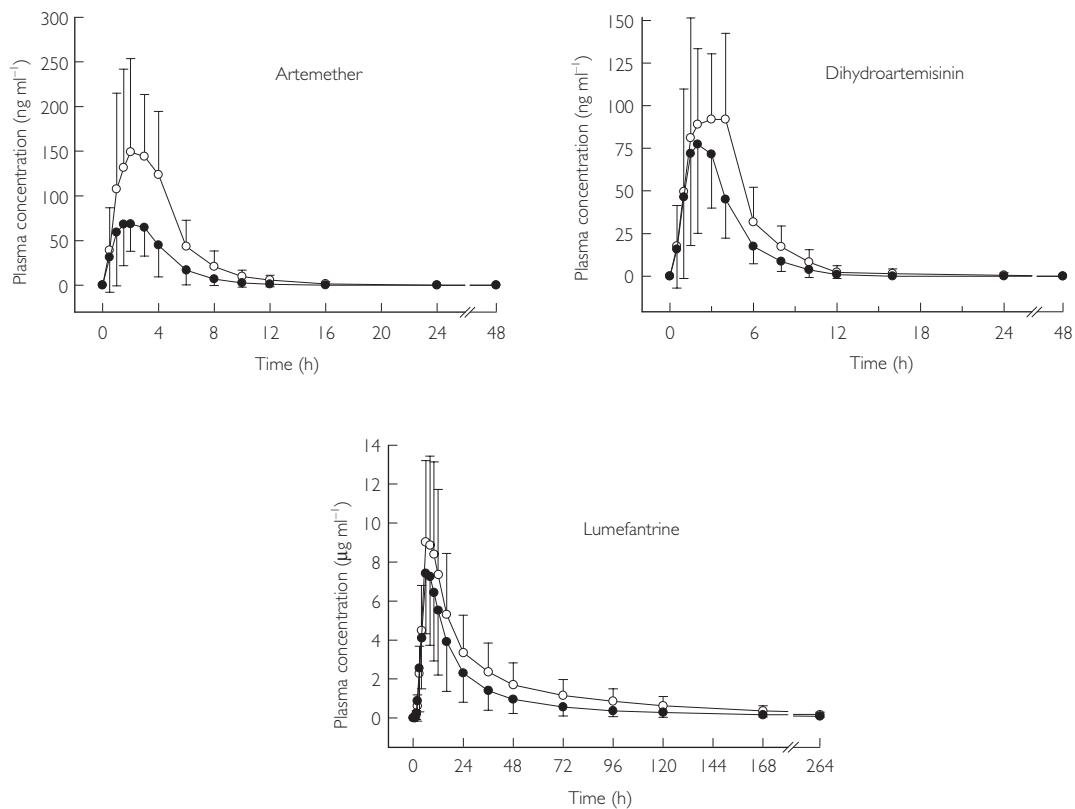
Lumefantrine

The mean plasma profiles of lumefantrine following the two treatments co-artemether alone or with ketoconazole are shown in Figure 1, and the pharmacokinetic parameters and statistical results are summarized in

Table 1 Mean ± s.d. ($n = 14$–15) pharmacokinetic parameters for artemether, dihydroartemisinin, and lumefantrine following administration of co-artemether alone or with ketoconazole.

	Co-artemether alone	Co-artemether + ketoconazole	Ratio[1] [90% CI]
Artemether			
C_{max} (ng ml^{-1})	104 ± 40	225 ± 77	2.24 [1.78, 2.83]
t_{max}[2] (h)	2.0 [1.0–4.0]	2.0 [1.0–4.0]	
AUC(0,last) (ng ml^{-1} h)	302 ± 135	718 ± 279	2.51 [2.07, 3.04]
AUC(0,∞) (ng ml^{-1} h)	320 ± 138	740 ± 286	2.39 [2.00, 2.86]
$t_{1/2}$ (h)	1.9 ± 0.8	2.5 ± 1.1	1.42 [1.12, 1.72]
Dihydroartemisinin			
C_{max} (ng ml^{-1})	104 ± 45	142 ± 55	1.40 [1.12, 1.74]
t_{max}[2] (h)	2.5 [1.0–4.0]	2.0 [1.0–4.0]	
AUC(0,last) (ng ml^{-1} h)	308 ± 112	475 ± 139	1.72 [1.42, 2.08]
AUC(0,∞) (ng ml^{-1} h)	331 ± 111	501 ± 155	1.66 [1.40, 1.98]
$t_{1/2}$ (h)	2.1 ± 0.9	3.1 ± 3.8	1.69 [0.02, 3.36]
Lumefantrine			
C_{max} (μg ml^{-1})	7.91 ± 3.49	10.1 ± 4.74	1.26 [0.96, 1.64]
t_{max}[2] (h)	6.0 [4.0–10.0]	6.0 [6.0–10.0]	
AUC(0,last) (μg ml^{-1} h)	195 ± 119	312 ± 181	1.66 [1.23, 2.23]
AUC(0,∞) (μg ml^{-1} h)	207 ± 123	333 ± 194	1.65 [1.23, 2.21]
$t_{1/2}$ (h)	95 ± 18	88 ± 15	0.93 [0.81, 1.04]

[1]median difference for t_{max}, CI = confidence interval, [2]median [range] for t_{max}.

Figure 1 Plasma concentrations (mean ± s.d.) of artemether, dihydroartemisinin, and lumefantrine following administration of co-artemether alone (●, $n = 14$) or in combination with ketoconazole (○, $n = 15$).

Table 1. Co-administration of co-artemether with keto-conazole was associated with an increase in lumefantrine exposure compared with co-artemether alone. AUC(0,∞) increased by 1.7 fold (from 207 to 333 µg ml^{-1} h) and C_{max} by 1.3 fold (from 7.91 to 10.1 µg ml^{-1}). No treatment difference was observed for either t_{max} (6.0 h following both treatments) or $t_{1/2}$ (95 *vs* 88 h). Although the 90% CIs for the ratios of AUC(0,last) and of AUC(0,∞) did not encompass 1, and therefore indicated that the extent of exposure from the two treatments was statistically different, the lower boundaries of the 90% CIs for the ratios of AUC and of C_{max} (for which the 90% CIs did encompass 1), were all below 1.25. Therefore, the drug–drug interaction between lumefantrine and keto-conazole was not considered statistically significant, although there was evidence of drug–drug interaction.

Discussion

The antimycotic agent ketoconazole is a potent inhibitor of hepatic and gastrointestinal cytochrome P450 isoenzyme CYP3A4, and of intestinal P-glycoprotein (P-gp), the multidrug transporter which pumps many drugs from cytoplasm back into the gastrointestinal tract [39, 40]. Co-administration of such potent inhibitors like keto-conazole with other drugs metabolized by CYP3A4 has been reported to lead to potentially highly clinically relevant interactions [22–27, 41]. Since artemether and lumefantrine are both predominantly metabolized by CYP3A4, this study in healthy volunteers aimed to investigate whether coadministration of ketoconazole could affect the pharmacokinetics of artemether, dihydroarte-misinin, and lumefantrine.

Since ketoconazole is a very potent CYP3A4 inhibitor, only a single dose of co-artemether was given in order to avoid any potential adverse effects of the latter. Hepatic CYP3A4 activity is nearly abolished after a single dose of ketoconazole [42], and since lumefantrine undergoes slow absorption with a 2 h lag time, pretreatment by ketoconazole prior to administration of co-artemether was not considered necessary. Treatment by ketoconazole for several days (after the dose of co-artemether) was considered to better distinguish between metabolism occurring in the liver compared to the intestine.

In the present study, coadministration of ketoconazole and co-artemether resulted in an increase in the exposure to the three active principles artemether, DHA, and lumefantrine. However, we did not consider the magnitude of this effect to be clinically important, because of the wide safety margin of co-artemether. The extent of

this change was within the between-subject variability in artemether, DHA, and lumefantrine pharmacokinetics observed in previous clinical trials, and, in addition, no relationship has so far been observed in the clinical setting between AUC (or C_{max}) and adverse effects [43]. Moreover, the influence of ketoconazole on artemether-lumefantrine exposure (1.3–2.5 fold increase) was much smaller than the 16-fold increase in plasma lumefantrine concentrations observed when co-artemether is given with food, as recommended [5, 34].

There are three possible mechanisms by which ketoconazole can increase exposure to artemether, DHA, and lumefantrine, namely inhibition of hepatic CYP3A4 resulting in reduction in systemic metabolism, inhibition of gastrointestinal CYP3A4 resulting in increased absorption, and/or inhibition of P-gp resulting in increased systemic bioavailability [44]. That the half-life of lumefantrine was not increased when co-artemether was given with ketoconazole suggests that the main effect of ketoconazole was to increase lumefantrine bioavalability through inhibition of presystemic CYP3A4 metabolism in the gut, rather than that in the liver. The results for artemether (i.e. increased AUC and $t_{1/2}$) suggest strongly that ketoconazole inhibits artemether metabolism mainly through its effect on hepatic CYP3A4. Similar data for dihydroartemisinin, although of smaller magnitude than these observed for its parent, artemether, provide evidence that dihydroartemisinin is also a substrate for CYP3A4, as reported for artemisinin [45].

These data demonstrate that potent inhibition of CYP3A4 activity has only a modest effect *in vivo* on the pharmacokinetics of artemether and lumefantrine, even though ketoconazole treatment was continued for several consecutive days. However, the present findings were not totally unexpected, since in addition to the predominant role of CYP3A4, *in vitro* data showed that CYP1A1, CYP1A2, CYP2B6, CYP2C9, and CYP2C19 may also contribute to the biotransformation of co-artemether [5, 6]. Our data are in agreement with a previous report showing that grapefruit juice, another inhibitor of CYP3A4 present in the mucosa cells of the gut wall, increased by nearly two-fold the systemic exposure to artemether after a dose of 100 mg [17].

The two treatments were safe and well tolerated in healthy subjects. Of particular importance is that no prolongations of the QTc interval were observed with co-artemether alone or in combination with ketoconazole, indicating that the observed increase in systemic exposure to artemether, DHA and lumefantrine had no detectable influence on the safety/tolerability profile of co-artemether.

All reports on co-artemether (Coartem®/Riamet®) show that the drug is safe and effective [16, 46, 47]. In contrast to several other antimalarials of the aryl amino alcohol class, co-artemether causes no cardiotoxicity, even when given in combination with quinine [16], or mefloquine [6, 32] an antimalarial known to enhance the QTc prolonging effect of halofantrine [48, 49]. QTc changes are common in malaria, and are variously ascribed to drug or disease effects [1]. A prospective population pharmacokinetic and pharmacodynamic analysis of lumefantrine in 266 Thai malaria patients showed that the drug had no effect on the QTc interval, and that there were no other adverse effects that could be ascribed unequivocally to the drug [43]. Similarly, lumefantrine was observed to have little or no effect on ventricular repolarization, suggesting that there is no *a priori* reason to withhold this drug from patients with a long QTc or any other cardiac abnormality [28].

In conclusion, overlapping therapy with the antimalarial artemether-lumefantrine and the potent CYP3A4 inhibitor ketoconazole is unlikely to increase the risk of adverse effects in the treatment of *Plasmodium falciparum* malaria. The pharmacokinetics of artemether and its active metabolite DHA, and lumefantrine were increased following the combined treatment with ketoconazole, but the difference was not considered to be of clinical importance. Artemether-lumefantrine alone had no effect on the ECG parameters, nor did the combination with ketoconazole. If these data in healthy subjects can be extrapolated to the clinical setting, no dose adjustment of co-artemether is recommended in malaria patients also receiving ketoconazole or other potent CYP3A4 inhibitors.

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