Ketoconazole increases plasma concentrations of antimalarial mefloquine in healthy human volunteers

W. Ridtitid MD FCFP, M. Wongnawa MSc, W. Mahatthanatrakul MD FCFP, N. Raungsri MSc and M. Sunbhanich PhD
Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand

SUMMARY

Background: Antimalarial mefloquine has a structure related to quinine. The major metabolite of quinine is 3-hydroxyquinine formed by cytochrome P450 3A4 (CYP3A4). Ketoconazole, a potent inhibitor of CYP3A4, is known to markedly increase plasma concentrations of various co-administered drugs including quinine.

Objective: To assess the effect of ketoconazole on plasma concentrations of mefloquine in healthy Thai male volunteers.

Methods: In an open, randomized two-phase crossover study separated by a 1-month period, eight healthy Thai male volunteers received a single oral dose of 500 mg mefloquine alone or co-administration with 400 mg/day ketoconazole orally for 10 days. Serial blood samples were collected at specific time points for a 56-day period. Plasma mefloquine and mefloquine carboxylic metabolite concentrations during 56 days were measured by a modified and validated high-performance liquid chromatographic method with UV detection.

Results: Co-administration with ketoconazole markedly increased the mean values of mefloquine AUC0–t, t1/2, and Cmax when compared with mefloquine alone by 79% (P < 0.001), 39% (P < 0.05) and 64% (P < 0.001) respectively. The AUC0–t, and Cmax of mefloquine carboxylic acid metabolite were decreased by 28% (P < 0.05) and 31% (P < 0.05), respectively when compared with mefloquine alone.

Conclusions: Co-administration with ketoconazole increased plasma mefloquine concentrations in healthy human volunteers. One of possible mechanisms of the increase in plasma mefloquine concentrations may be the result of the inhibition of CYP3A4 by ketoconazole. In case of mefloquine is co-administered with ketoconazole, drug–drug interactions should be recognized and the dose of mefloquine should be adjusted to maximize the therapeutic efficacy and to reduce the cost of therapy.

Keywords: drug–drug interactions, ketoconazole, mefloquine, pharmacokinetics, plasma concentrations

INTRODUCTION

Mefloquine [dl-erythro-a-(2-piperidy1)-2,8-bis (trifluoromethyl)-4-quinoline methanol] is a quinolinemethanol antimalarial drug structurally related to quinine. It is an effective single dose therapy for all species of malarial parasites infecting humans, including multidrug-resistant Plasmodium falciparum. It is still used both in prophylaxis and treatment of the disease in most areas with multidrug-resistant P. falciparum (1–3). Mefloquine is relatively well tolerated and has the advantage of a single daily dose regimen making it suitable for prophylactic use (4). However, mefloquine monotherapy for uncomplicated falciparum malaria was discontinued and replaced with a combination of mefloquine (25 mg/kg) and artesunate administration (4 mg/kg/day) (2, 5). Mefloquine is distributed extensively in tissues and eliminated slowly, with considerable differences between individuals (2). Following the oral administration of a single 25 mg/kg dose of mefloquine to patients with acute falciparum, the mean values of Cl/f, Vd/f, Ke, t1/2 and AUC0–t of mefloquine were 0.733 L/kg/day, 20.37 L/kg, 0.036/day, 19.3 days and 34.106 ng/mL/day, respectively (6). After 1000 mg
(divided into three doses over 12 h) mefloquine administration orally in healthy White male, the mean ± SD values of $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{0-846 \, h}$, $AUC_{0-\infty}$ and $t_{1/2}$ were 1000 ± 266 ng/mL, 23 ± 10 h, 280 ± 107 µg/mL/h, 375 ± 125 µg/mL/h and 427 ± 198 h, respectively (7). Two mefloquine metabolites identified in humans are hydroxy and carboxylic acid metabolites. The main metabolite is 2,8-bis-trifluoromethyl-4-quinolinecarboxylic acid and inactive for *P. falciparum* (8). Quinine is a widely used antimalarial drug for the treatment of severe or multidrug-resistant *P. falciparum* (9, 10). The CYP3A4 is a major cytocrome P450 involved in the metabolism of quinine both in *vitro* and *in vivo* (6, 11, 12).

Ketoconazole, a broad spectrum azole antifungicotic, is a potent inhibitor of CYP3A4 resulting the significant increase in plasma concentrations of various drugs co-administered, for example quinine (13). As ketoconazole is one of azole compounds, a number of side-effects are associated with ketoconazole as a result of inhibition of these mammalian enzymes (14). Ketoconazole leads to liver damage because of its ability to inhibit CYP3A4, the major P450 isofrom of the liver (15). The inhibition of CYP3A4 results in drug–drug interactions involving ketoconazole and a decrease in the rate of clearance of many drugs (16). Steroid biosynthesis by P450 enzymes is also inhibited by ketoconazole, presumably because of the binding of ketoconazole to the mitochondria P450 enzymes, and the administration of low doses of ketoconazole leads to a significant reduction in serum androgen levels (17).

Mefloquine has a structurally chemical related to quinine. As quinine is extensively metabolized by CYP3A4 to form 3-hydroxyquarine, a major metabolite (6, 11, 12), therefore, ketoconazole would theoretically alter the metabolism of mefloquine.

**METHODS**

**Subjects**

Eight Thai male volunteers, age 16–39 years (mean age 29.5 ± 8.4 years) and weighed 56–64 kg (mean weight 61.5 ± 2.6 kg) participated in the study. Prior to the study, a medical history, physical examination, standard biochemical and haematological screening test (CBC, FBS, BUN, creatinine, SGOT, SGPT, direct bilirubin and albumin/globulin) were carried out in each volunteer. None of volunteers was a smoker or used continuous medications. Drinking of alcoholic beverages, coffee and tea were not allowed at least 1 month prior to and during the entire period of study. Written informed consent was received from each subject prior to the study. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Hat Yai, Thailand.

**Study protocol**

The study was an open-label, two-phase crossover design with a 1-month separation between phases. A single oral dose of 500 mg mefloquine (MEQUIN®, 250 mg/tablet, Lot No.010192; Atlantic Laboratories Corp. Ltd, Bangkok, Thailand) was kindly donated by the Insect Prevention Center, Songkhla, Thailand.

**Phase 1.** On the study day, four subjects ingested only 500 mg mefloquine with 200 mL water. Another four subjects received mefloquine plus 400 mg ketoconazole (KETAZOL, 200 mg/tablet, Lot No.1A918/31; Central Poly Trading Co. Ltd, Bangkok, Thailand). Each subject ingested 400 mg ketoconazole once daily before breakfast for 5 days prior to mefloquine administration and for a further 5 days.

**Phase 2.** The four subjects who ingested 500 mg mefloquine alone in phase 1 or treatment 1 were changed to have mefloquine plus ketoconazole, and another four subjects who ingested mefloquine plus ketoconazole in phase 1 or treatment 1 were changed to have only mefloquine alone.

All subjects fasted overnight before mefloquine administration and received a regular meal 3 h after mefloquine. The subjects were not allowed to smoke or have coffee, tea, alcohol or cola on the test day.

**Determination of plasma mefloquine and its carboxylic acid metabolite**

A forearm vein was inserted with a sterile intravenous catheter and for the collection of blood samples, maintained patent with 1 mL of a dilute heparin solution (100 unit/mL) after each
sampling. Serial venous blood samples (5 mL) were collected into heparinized tubes before drug administration and at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 h, and 2, 3, 4, 7, 14, 21, 35, 49 and 56 days post-drug administration. Samples were centrifuged not later than 30 min after collection, and the plasma was separated and stored at −60 °C until analysis. The plasma mefloquine (molecular weight 414.79) and mefloquine carboxylic acid metabolite (molecular weight 309.13) concentrations were measured by a high-performance liquid chromatographic (HPLC) method (18, 19). The limit of quantification of mefloquine and its carboxylic acid metabolite was 62.5 ng/mL. The intraday coefficient of variation of both mefloquine and its carboxylic acid metabolite was 1.60–9.07%, whereas the interday coefficient of variation was 3.51–10.21%. The relative recovery of standard mefloquine and its carboxylic acid metabolite in human plasma was 83–98% and 89–100% respectively.

Pharmacokinetic calculations

The pharmacokinetic parameters were analysed using a one-compartmental model and WinNonlin version 4.1 (Pharsight, Mountain View, CA, USA). The total area under the plasma concentration–time curve (AUC) was calculated by the linear trapezoidal rule. The elimination rate constant (\( K_e \)) was estimated from the least-squares regression slope of the terminal plasma concentrations time course. The half-life (\( t_{1/2} \)) of mefloquine and mefloquine metabolite were calculated using the following equation

\[
    t_{1/2} = \ln 2 / K_e
\]

The maximum plasma concentration (\( C_{\text{max}} \)) and the time to reach \( C_{\text{max}} \) (\( T_{\text{max}} \)) were obtained from the plasma concentration–time data.

Statistical analysis

All results were expressed as mean ± standard deviation (SD). Differences in mefloquine and mefloquine metabolite pharmacokinetic parameter among control and treatment groups were tested by one-way ANOVA with \( P < 0.05 \) taken as the level of significance. The effect of period, sequence and interaction were evaluated with the use of two-way ANOVA analysis.

RESULTS

Adverse effects

Eight healthy volunteers were enrolled and completed this study. No side-effects were observed after taking 500 mg of mefloquine alone. However, two subjects reported mild headache during ketoconazole co-administration. This symptom occurred only for a few days, and did not require any specific treatment. No significant laboratory abnormalities occurred in the subjects, and physical examinations revealed no abnormal findings at the end of the study.

Pharmacokinetics

The mean plasma concentration–time profiles of mefloquine and of its carboxylic acid metabolite after mefloquine administration alone and co-administered with ketoconazole are shown in Fig. 1 and the pharmacokinetic parameters are summarized in Table 1.

The mean \( \text{AUC}_{0-t} \), \( t_{1/2} \), and \( C_{\text{max}} \) values of mefloquine co-administered with ketoconazole increased by 79% (159.66 ± 33.28 vs. 286.05 ± 64.25 mg/L/h; \( P < 0.001 \)), 39% (322.68 ± 99.95 vs. 448.41 ± 103.88 h; \( P < 0.05 \)) and 64% (345.10 ± 43.22 vs. 567.65 ± 88.69 ng/mL; \( P < 0.001 \)), respectively when compared with mefloquine alone. The mean \( \text{AUC}_{0-t} \) and \( C_{\text{max}} \) of mefloquine carboxylic acid metabolite decreased by 28% (492.43 ± 141.66 vs. 352.29 ± 47.08 mg/L/h; \( P < 0.05 \)) and 31% (606.11 ± 184.00 vs. 419.65 ± 45.02 ng/mL; \( P < 0.05 \)), respectively. The \( t_{1/2} \) value of the carboxylic acid metabolite decreased by 15% (679.08 ± 358.49 vs. 575.03 ± 82.28 h; \( P > 0.05 \)) but was not significantly different to the control values. The mean \( T_{\text{max}} \) values for mefloquine and mefloquine carboxylic metabolite after co-administration of mefloquine with ketoconazole were not significantly different from seen with mefloquine alone.

DISCUSSION

These results suggest that co-administered ketoconazole increased the plasma concentration of mefloquine. The increase in \( \text{AUC}_{0-t} \) (79%) and \( C_{\text{max}} \) (64%) of mefloquine was likely the result of decreased presystemic metabolism of mefloquine. The rate of absorption of mefloquine was unlikely
to have been affected as there was no significant difference in the $T_{\text{max}}$ of mefloquine with and without ketoconazole. The presystemic metabolism of mefloquine probably involved intestinal and hepatic CYP3A4. The increase in elimination $t_{1/2}$ of mefloquine in subjects with ketoconazole treatment indicated an increased systemic metabolism of mefloquine. In addition, the $AUC_{0-\infty}$ and $C_{\text{max}}$ of mefloquine carboxylic acid metabolite were significantly reduced with ketoconazole co-administration. Reduced presystemic mefloquine metabolism is reflected in a decreased rate of metabolite formation. Ketoconazole appears to have reduced the metabolism of mefloquine during both presystemic and elimination phases (increased $C_{\text{max}}$ and longer $t_{1/2}$ values for mefloquine when given with ketoconazole). The liver and intestine play an important role in the presystemic metabolism of many CYP3A4 substrates, and ketoconazole is a potent inhibitor of CYP3A4 in these organs. Therefore, the increased mean $AUC_{0-\infty}$, $C_{\text{max}}$ and $t_{1/2}$ of mefloquine after single oral dose administration with ketoconazole for 10 days may be the result of inhibition of CYP3A4 by ketoconazole, which were similar to those occurring with quinine (12) as mefloquine has a chemically structure related to quinine. Significant quantities of CYP3A4 are found in small bowel enterocytes and liver (20). CYP3A4 is the most abundantly expressed CYP

Table 1. Pharmacokinetic parameters (mean ± SD) of mefloquine and its carboxylic acid metabolite in eight subjects following a single oral of dose 500 mg mefloquine alone or in combination with oral administration of 400 mg/day ketoconazole for 10 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mefloquine alone (control phase)</th>
<th>Mefloquine + ketoconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0-\infty}$ (mg/L/h)</td>
<td>159.66 ± 33.28</td>
<td>286.05 ± 64.25**</td>
</tr>
<tr>
<td>% of control (range)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>322.68 ± 99.95</td>
<td>448.41 ± 103.88*</td>
</tr>
<tr>
<td>% of control (range)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>17.99 ± 8.17</td>
<td>12.36 ± 3.00</td>
</tr>
<tr>
<td>% of control (range)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>345.10 ± 43.22</td>
<td>567.65 ± 88.69**</td>
</tr>
<tr>
<td>% of control (range)</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD. $AUC$, area under the plasma concentration–time curve; $t_{1/2}$, elimination half-life; $T_{\text{max}}$, time to reach $C_{\text{max}}$; $C_{\text{max}}$, maximum plasma concentration. Data are mean ± SD.

*p < 0.05, **p < 0.001 significantly different compared with control phase (one-way ANOVA).
and accounts for approximately 30–40% of the total CYP contents in human adult liver and small intestine (21). As ketoconazole is a potent inhibitor of CYP3A4 in both liver and small bowel enterocytes it provides the obvious explanation for the strong interaction observed. We previously reported that cimetidine, a potent CYP3A4 inhibitor, reduced the clearance, and prolonged the elimination $t_{1/2}$ of mefloquine in a similar manner to quinine (22). Ketoconazole is also a potent P-glycoprotein (P-gp) inhibitor, thereby decreasing P-glycoprotein-mediated drug transport and metabolism (23). In animal studies co-administration of ketoconazole (50 mg/kg, i.v.) caused an eightfold increase in brain level of nelfinavir and a 3.5-fold increase in plasma concentration in mice (24). Both CYP3A4 and P-gp have broad substrate specificity. Therefore, there is striking overlap of substrate between CYP3A4 and P-gp. Because of overlapping substrate specificity, and because of co-expression of CYP3A enzymes and P-gp in the intestine, kidney and liver, it is conceivable that P-gp may play an important role in drug absorption, by limiting drug transport from the intestinal lumen and metabolism (23). The fact that inhibition of P-gp by ketoconazole contributed to the observed interaction with mefloquine, cannot be excluded in this study. The higher $t_{1/2}$ in subjects with ketoconazole co-administration indicated a decreased hepatic metabolism of mefloquine. This suggests that ketoconazole inhibits the hepatic CYP3A4-mediated metabolism of mefloquine.

The significant decrease in the AUC$_{0-1}$ and $C_{\text{max}}$ of mefloquine carboxylic acid metabolite after co-administration of ketoconazole is probably the result of the cytochrome P450 by the latter. In support, the $t_{1/2}$ value of mefloquine carboxylic acid metabolite after ketoconazole co-administration decreased by 15% compared with mefloquine alone. Differences in drug metabolism and their determinants in human organisms have been intensively investigated over the years. In general, genetic factors (polymorphism) are more important than environmental ones. It was reported that CYP3A4*1B carriers required more tacrolimus to reach target trough concentrations compared with CYP3A4*1 homozygotes (25). However, among the latter, age, nutrition, disease and drug interaction were common factors altering drug metabolism. In addition, renal elimination of either drug or metabolite is subject to several factors such as urine pH and changes in renal blood flow.

Plasma concentrations of 200–300 ng/mL may be necessary to achieve chemosuppression in P. falciparum infections. Plasma mefloquine concentrations from volunteers experiencing prophylaxis failure were all less than 400 ng/mL, suggesting that higher mefloquine concentrations are necessary to suppress P. falciparum (18). In this study, maximum plasma concentration of mefloquine after mefloquine administration alone and co-administered with ketoconazole were $345.10 \pm 43.22$ ng/mL and $567.65 \pm 88.69$ ng/mL respectively. Therefore, our results indicated that ketoconazole (400 mg for 10 days) raised plasma concentrations of mefloquine sufficiently for a schizontocidal activity against P. falciparum. This effect is beneficial in subjects who would otherwise receive subtherapeutic doses of mefloquine.

**CONCLUSIONS**

Ketoconazole enhances plasma concentrations of mefloquine considerably by inhibiting its metabolism in the liver rather than the small intestine. Inhibition of CYP3A4-mediated metabolism, is a likely explanation. Both mefloquine and ketoconazole are widely prescribed in some countries. Thus, clinicians should be aware of this interaction.

**ACKNOWLEDGEMENTS**

This study was supported by grants from the Thai Government, Faculty of Science, Graduate Studies, Prince of Songkla University, Thailand. We thank F-Hoffmann-La Roche, Basel, Switzerland, for donating standard mefloquine hydrochloride and its carboxylic acid metabolite for use in HPLC analysis.

**REFERENCES**


with acute falciparum malaria. *Clinical Pharmacology and Therapeutics, 66*, 472–484.


