Effects of Co-trimoxazole Co-administration on the Pharmacokinetics of Amodiaquine in Healthy Volunteers

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INTRODUCTION

Cytochrome P450 (CYP) enzymes represent a family of proteins that contribute predominantly to the metabolism of drugs and other xenobiotics. Alteration in the activity of these enzymes in vivo represents the major underlying mechanism behind pharmacokinetic drug-drug interactions [1]. In humans, some CYP enzymes have been designated as more predominant in the metabolism of drugs than others. These include CYP1A2, 2C9, 2C19, 2D6, 2E1 and 3A4. These six enzymes have been studied extensively with regard to their role in drug metabolic clearance as well as drug-drug interactions [2].

However; other investigations have uncovered roles for other CYP enzymes, such as CYP2C8, in the metabolism of some drugs. Thus, CYP2C8 can also be the target of drug-drug interactions for those drugs in which it plays a predominant role in the clearance [3]. Drug interactions that arise by inhibition of CYP2C8 have been described, including interactions between cerivastatin and gemfibrozil [4], repaglinide, and gemfibrozil [5], and repaglinide and trimethoprim [6].

Amodiaquine (AQ), 4-(7-chloro-4-quinolylamino)-2-(diethylaminomethyl) phenol dihydrochloride is a 4-aminoquinoline antimalarial drug which acts by inhibiting the degradation of hemoglobin in the food vacuole of plasmodium parasite [7]. After oral administration, AQ undergoes rapid and extensive hepatic metabolism by N-dealkylation to the active metabolite, N-desethylamodiaquine (DEAQ) [8], [9]. This study evaluates the effect of CTZ co-administration on the pharmacokinetics of AQ.

Methods: In an open, two-way crossover study, 16 healthy volunteers were randomized to receive 600 mg single oral dose of AQ with or without the eleventh dose of CTZ (960 mg every 12 h for 7 days.) Blood samples were collected at pre-determined time intervals and analyzed for AQ and its major metabolite, DEAQ using a validated HPLC method.

Results: Co-administration of AQ and CTZ resulted in significant increases in the total area under the concentration–time curve (AUC∞), maximum plasma concentration (Cmax) and terminal elimination half-life (T½) of AQ compared with values with AQ dosing alone (AUC∞: 234.36±57.21 vs 366.42±62.48 ng/ml; Cmax: 40.28±11.15 vs 49.1±35.6 ng/ml; T½: 9.24±2.97 vs 6.49±3.56 h), while the oral plasma clearance markedly decreased (2654.28±650.12 L/h) vs 3862.66±756.38 L/h), Co-administration also led to a pronounced decrease in the ratio of AUC (metabolite)/AUC (unchanged drug) and highly significant decreases in Cmax and AUC of the metabolite.

Conclusion: Study evaluated for the first time the effect of CTZ co-administration on the pharmacokinetics of AQ in healthy adult volunteers. CTZ significantly increased AQ exposure and decreased plasma levels of the active metabolite DEAQ.

Keywords: Amodiaquine, Desethylamodiaquine, Co-trimoxazole, Pharmacokinetics, CYP2C8.
minor metabolites being 2-hydroxyl-DEAQ and N-bisDEAQ [9, 13]. Although the formation of DEAQ is rapid, its elimination is very slow with a terminal half-life of over 100 hours. Both AQ and DEAQ possess antimalarial activity with AQ being 3 times more potent than DEAQ [9, 13]. Because AQ is rapidly cleared and the formed DEAQ retains high plasma concentrations for a long time, AQ is considered a pro drug, which is bioactivated to DEAQ [9, 13].

Co-trimoxazole, [CTZ] a combination of sulfamethoxazole and trimethoprim, is an inhibitor of bacterial purine biosynthesis and is commonly used to treat HIV-associated Pneumocystis jiroveci infections [14]. The standard treatment dose of CTZ (960 mg 12 hourly) has been reported to interfere with the elimination of several drugs by various mechanisms. Competitive inhibition with trimethoprim for the human organic cation transporter (b01CT) has been implicated in the decreased renal tubular secretion of the nucleotide reverse transcriptase inhibitors (NRTI) zidovudine and lamivudine [15,16], whereas a reversible increase in serum creatinine has been reported during concomitant treatment with cyclosporin and CTZ in renal transplant patients [17]. In vitro data suggest that at clinical doses, sulfamethoxazole and trimethoprim selectively inhibit the CYP isoenzymes CYP2C9 and 2C8 respectively and lose their potential for a pharmacokinetic interaction between these two agents. However, the magnitude and clinical significance of such an interaction can only be evaluated through studies. Patients receiving treatment with CTZ for susceptible infections may require concomitant treatment with AQ for malaria infection. There is very sparse information on interaction between CTZ and antimalarial drugs, and there is no published data on pharmacokinetic interaction between CTZ and AQ. The aim of the present study therefore, was to determine the effect of steady state CTZ on the pharmacokinetics of AQ in healthy volunteers.

MATERIALS AND METHODS

Chemicals and reagents

AQ dihydrochloride and DEAQ dihydrochloride were obtained from Parke-Davis, U. S. A., and quinidine from BDH Laboratory Supplies, Poole, England. AQ dihydrochloride tablets (Parke-Davis, U. S. A.) were purchased from a retail pharmacy in Nigeria. HPLC grade acetonitrile and methanol, and analytical grade diethyl ether, perchloric acid, sodium hydroxide and hydrochloric acid were purchased from Sigma (Sigma-Aldrich chemical company, Germany).

Instrument and chromatographic conditions

The High Performance Liquid Chromatography (HPLC) system consisted of Agilent fitted automatic injector system fitted with a variable UV detector. The stationary phase was a reversed-phase C18 column [Zorbax Eclipse C18-3.5 µm (200 x 4.6 mm I.D.)]. The solvent system for HPLC consisted of acetonitrile: 0.02 M potassium dihydrogen phosphate (10:90). The pH of the mobile phase was adjusted to 40 with orthophosphoric acid. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min. The experiments were performed at ambient temperature. The method was a slight modification of Gita et al. (2004) [19]. Wärtsilä (Fissions), precision pipettes (MLA), table centrifuge (Gallenkamp) and digital sonicator (Gallenkamp) were used for the extraction procedure.

Analytical procedure

To 1 ml of plasma placed in a 15-ml screw capped extraction tube, were added 20 µl of 500 µg/ml quinidine solutions (internal standard) and 2 ml of acetonitrile before mixing for about 15 seconds, followed by mechanical tumbling for 15 min. After centrifuging for 10 min at 3000 g, the liquid phase was transferred to a clean tube, to which was added 2 ml of ammonia. The mixture was then extracted by mechanical tumbling for 15 min, with 2 x 5 ml of diethyl ether. After centrifugation and separation, the combined organic phases were evaporated to dryness and the residue was reconstituted in 100 µl of methanol while a 50 µl aliquot was injected onto the HPLC column. Calibration curve based on peak area ratios was prepared by spiking drug-free plasma with standard solutions of AQ and DEAQ to give concentration ranges of 2–30 ng/ml and 20–300 ng/ml respectively. The samples were taken through the extraction procedure described above.

Subjects

Twelve males and four females adult Nigerians, aged between 30 and 38 years and weighing between 64 and 75 kg took part in the study. They were all judged healthy by a physician on the basis of medical history, clinical examination, biochemical, and hematological screening prior to entry into the study. Subjects were excluded from participating if they met none of the following criteria: pregnancy, breast feeding, history of hypersensitivity reactions to AQ or similar agents (Chloroquine, quinidine, quinolones), use of any medications that could potentially interact with the study drug, any liver function test more than three times the upper limit of normal, or evidence by history or physical examination of gastrointestinal, psychiatric, cardiovascular or neurological disorders. All participants gave written informed consent, and approval was obtained from the Research Ethics Board and safety Committee of the College of Health Sciences, Osun State University, Osogbo.

Study design, drug administration and sample collection

The study was an open-label, crossover pharmacokinetic study. A single oral dose of 600 mg AQ dihydrochloride tablets was given to each of the sixteen volunteers with and without the eleventh dose of 960 mg CTZ (given 12 hourly) in a crossover design after an overnight fast. 5 ml blood samples were withdrawn by venipuncture from the forearm of each subject prior to and at 0.08, 0.25, 0.5, 1.5, 3, 5, 24, 48 and 96 hours after drug administration into heparinized tubes. They were immediately centrifuged (3000 g at 20 °C for 10 min) to separate plasma. The plasma aliquots were stored at -20 °C until analyzed. The plasma samples were analyzed for AQ and DEAQ to obtain their baseline pharmacokinetics and then to evaluate the effect of CTZ on the pharmacokinetics of AQ.

Pharmacokinetic evaluation

The pharmacokinetic (PK) parameters for AQ and DEAQ were calculated with the computer program WinNonLin (version 1.5). The data were analyzed using non-compartmental analysis. The parameters that could be established were as follows: time point of maximum observed concentration in plasma (Tmax), concentration in plasma corresponding to Tmax (Cmax), terminal half-life (T1/2), area under the plasma concentration versus time (C–t) curve (AUC(0–t)). The terminal half-life was calculated from the terminal elimination rate constant: k. This rate constant was calculated as means of linear regression of the final part of the ln C-t curve. The final half-life could be calculated by T1/2 = ln (2)/k. For the calculation of the elimination rate constant, we used the three final concentrations of the C-t curve (24, 48, and 96 h). The AUC(0–t) was calculated by the logarithmic trapezoidal rule.

Statistical evaluation was performed with the statistical program SPSS 7.0. Direct comparisons of the raw data were performed using the two-sided paired t test. The Wilcoxon matched pairs signed ranked test was used to evaluate the difference between any pair of data, i.e. effects of CTZ on the pharmacokinetics of AQ or on DEAQ disposition. Results were recorded as mean±SD. In all, a value of P<0.05 was considered statistically significant.

RESULTS

All subjects tolerated the 600 mg single oral dose of AQ. The most frequently reported adverse event was mild nausea (all subjects). Mild abdominal pain occurred in a further five subjects. There were no serious adverse events during the study and no withdrawal due to adverse events or for any other reason. The extraction procedure employed in this study produced clean and clear supernatants from plasma as there was no interference from endogenous compounds. AQ was well resolved from its major metabolite and from the internal standard. The limit of quantification for AQ and DEAQ were 0.5 ng/ml and 10 ng/ml respectively. The pharmacokinetic parameters obtained following administration of AQ alone or with CTZ are presented in table 1.
Co-administration of CTZ was associated with marked increases (P<0.05) in the C\text{max}, AUC, and elimination T\text{1/2} of AQ increased by 62% [95% CI 55, 70], 37% [95% CI 35, 42], and 56% [95% CI 60, 65], respectively, in the presence of CTZ. Also, the oral plasma clearance (CL/F) of AQ was highly reduced with CTZ co-administration by about 31% [95% CI 28, 34]. The pharmacokinetic parameters of DEAQ following administration of AQ, with and without CTZ, are also shown in table 1. The C\text{max}, T\text{max}, and AUC\text{0–96h} of the metabolite were significantly diminished in the presence of CTZ (P<0.05). The C\text{max} reduced by 25% [95% CI 21, 28], T\text{max} reduced by 33% [95% CI 30, 35], while AUC\text{0–96h} reduced by 12% [95% CI 10, 14].

Co-administration of CTZ also resulted in a pronounced decrease in the ratio of the AUC of AQ metabolite to that of unchanged drug (i.e. metabolic ratio, MR) by about 44% [95% CI 40, 48]. There was no significant change (P>0.05) in the Tmax of the metabolite. There were no significant differences between the treatment groups regarding adverse events, vital signs, and laboratory tests, (data not shown).

**DISCUSSION**

This study was performed to evaluate the effect of CTZ co-administration on the pharmacokinetics of AQ, a 4-aminopyrimidine antimalarial drug. Previous reports have shown that AQ is well absorbed from the gastrointestinal tract with a T\text{max} in the range of 2.7–3.0 h. Its elimination half-life is approximately 5 h and is rapidly cleared by extensive metabolism followed by excretion of metabolites in the urine [20]. The results from the present study indicate that the pharmacokinetic parameters obtained for AQ when administered alone, such as T\text{max}, elimination T\text{1/2}, CL/F, and AUC, are generally in agreement with the values obtained in other single dose pharmacokinetic studies [20-22]. With CTZ co-administration, the observed marked increase in the T\text{max} of AQ (table 1) which is indicative of a slower rate or prolongation of absorption of the antimalarial may be attributed to the modulation of intestinal P-glycoprotein by CTZ. It has been demonstrated that CTZ is not a P-glycoprotein substrate but can slightly induce P-glycoprotein functionality and expression probably through induced cell stress [23].

Since AQ is a substrate for P-glycoprotein [24] it is possible for its absorption to be prolonged by P-glycoprotein up-regulation caused by CTZ. This speculation is based on reports indicating that drug-induced increase in P-glycoprotein expression can result in prolongation of T\text{max} of a co-administered drug. For example, the T\text{max} of levofloxacin was prolonged by 50% following efavirenz co-administration and this was ascribed to up-regulation of P-glycoprotein induced by efavirenz [25]. Moreover, in our previous study, the T\text{max} of prougnil was prolonged significantly following concomitant administration with efavirenz and this was ascribed to up-regulation of P-glycoprotein induced by efavirenz [26]. The total systemic exposure (AUC) of AQ was substantially increased (mean of about 80%) in the presence of CTZ (table 1) and this is quite evident in the significant difference in the plasma concentration profiles of AQ with or without CTZ administration (fig. 2A).

The increased systemic drug exposure coupled with the markedly diminished oral drug clearance (CL/F) and significantly prolonged elimination T\text{1/2} of AQ suggests a systemic inhibition of metabolism of the drug by CTZ. This assertion is buttressed by the observation of an evident marked reduction in plasma levels of the major

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**Table 1: Effect of co-administration of CTZ on the pharmacokinetic parameters of AQ and DEAQ**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AQ alone</th>
<th>AQ with CTZ</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\text{max} (ng/ml)</td>
<td>24.86±7.28</td>
<td>40.28±11.15</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T\text{max} (h)</td>
<td>2.80±1.71</td>
<td>3.86±2.14</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AUC (ng/ml)</td>
<td>234.36±57.21</td>
<td>366.42±62.48</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T\text{1/2} (h)</td>
<td>6.49±3.56</td>
<td>9.24±2.97</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>C\text{24h} (ng/ml)</td>
<td>5.23±1.25</td>
<td>6.1±1.42</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>366.2±67.56</td>
<td>265.2±65.12</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>V\text{F} (L)</td>
<td>607.5±41.25</td>
<td>6496.3±234.27</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>DEAQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\text{max} (ng/ml)</td>
<td>560.28±102.16</td>
<td>420.16±98.27</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T\text{max} (h)</td>
<td>4.62±1.54</td>
<td>4.5±2.16</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>T\text{1/2} (h)</td>
<td>189.23±61.24</td>
<td>126.52±3.37</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AUC\text{0–24h} (ng/ml)</td>
<td>185.60±62.14</td>
<td>16218.7±620.44</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>C\text{24h} (ng/ml)</td>
<td>85.4±61.28</td>
<td>70.24±14.19</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>MR</td>
<td>79.19±3.56</td>
<td>44.26±2.68</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

C\text{max}: peak plasma concentration, T\text{max}: time to C\text{max}, AUC: area under the concentration–time curve, T\text{1/2}: terminal half-life, CL/F: apparent oral clearance, V\text{F}: apparent volume of distribution, MR: metabolic ratio.
metabolite (DEAQ) ([fig. 2B]), which is reflected in significant decreases in the C_{max} and AUC of the metabolite. Previous studies have shown that both CYP2C8 and CYP3A4 contribute to the metabolism of AQ but the former is the major contributor to the biotransformation [27, 28]. Since trimethoprim, a component of CTZ, has been demonstrated as an inhibitor of CYP2C8 as well as a mixed inducer/inhibitor of CYP3A4 [18], the increase in plasma levels of AQ following co-administration with CTZ is most likely due to the inhibition of CYP2C8 and probably a contribution from CYP3A4 inhibition.

In our previous study looking at AQ pharmacokinetics following co-administration with 600 mg efavirenz once daily, efavirenz significantly increased AQ C_{max} and AUC; [29]. Table 1 shows a pronounced decrease (44%) in the ratio of AUC of metabolite to that of unchanged drug (MR). This further strengthens the point that a metabolic interaction occurs between AQ and CTZ, and that CTZ inhibits the metabolism of AQ. The increased plasma levels of AQ as the result of inhibition of its metabolism by the co-administered CTZ may increase the risk of hepatotoxicity and agranulocytosis adverse events of AQ. After oral administration, AQ is rapidly absorbed from the gastrointestinal tract. In the liver, it undergoes rapid and extensive metabolism to DEAQ which concentrates in blood cells [11, 13]. AQ is three times more potent than DEAQ but the concentration of AQ in blood is quite low [11, 12]. Therefore, DEAQ is responsible for most of the observed antimalarial activity, and inhibition of AQ metabolism by co-administered CTZ, also decreases plasma levels of DEAQ which may also lead to a decreased antimalarial protection.

This study was carried out in healthy volunteers, and it is pertinent to note that changes in AQ pharmacokinetics occur in patients with malaria. In malaria, there is an elevated level of α-1-acid glycoprotein that is proportional to the severity of the infection. The raised concentrations of this protein account for increased plasma protein binding of AQ with resultant increased plasma AQ levels in malaria [24]. Since the unbound fraction of AQ in plasma is lower in patients with malaria, the degree of interaction between the drug and CTZ in these patients might be different from that observed in healthy subjects.

CONCLUSION

This study evaluated for the first time the effect of CTZ co-administration on the pharmacokinetics of AQ in healthy adult volunteers. CTZ significantly increased AQ exposure and decreased plasma levels of the main active metabolite, DEAQ. Consequently, the toxicity of AQ and antimalarial protection of DEAQ may be increased and decreased respectively when CTZ is co-administered with AQ. More studies are needed to further evaluate impact of this interaction and of CYP2C8 genetic polymorphisms on the clinical outcomes of treatment.

CONFLICT OF INTERESTS

Declared None

REFERENCES