Concurrent administration of donepezil HCl and ketoconazole: assessment of pharmacokinetic changes following single and multiple doses

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Aim The aim of this study was to examine the pharmacokinetics of donepezil HCl and ketoconazole separately, and in combination, following administration of single and multiple oral doses.

Methods This was an open-label, randomized, three-period crossover study in healthy volunteers (n = 21). During each treatment period, subjects received single daily doses of either donepezil HCl (5 mg), ketoconazole (200 mg), or a combination of both drugs for 7 consecutive days. Pharmacokinetic comparisons were made between treatment groups for the day 1 and day 7 profiles. Each treatment period was followed by a 3-week, drug-free washout period.

Results On both day 1 and day 7, a statistically significant difference was observed between the donepezil and the donepezil + ketoconazole treatment groups in terms of C_max and AUC(0–24) of donepezil. The concurrent administration of both drugs resulted in a 12% greater C_max (9.5 ng ml\(^{-1}\) versus 8.4 ng ml\(^{-1}\); \(P = 0.01\)) and a 12% greater AUC(0–24) (135.2 ng h ml\(^{-1}\) versus 118.7 ng h ml\(^{-1}\); \(P = 0.001\)) than donepezil alone on day 1, and a 26.8% greater C_max (97.7 ng ml\(^{-1}\) versus 27.6 ng ml\(^{-1}\); \(P < 0.0001\)) and a 26.4% greater AUC(0–24) (680.9 ng h ml\(^{-1}\) versus 501.0 ng h ml\(^{-1}\); \(P < 0.0001\)) than donepezil alone on day 7.

In contrast, ketoconazole plasma concentrations were unaffected by the concurrent administration of donepezil, and there were no statistically significant differences in ketoconazole pharmacokinetics when ketoconazole administered alone was compared with ketoconazole administered with donepezil.

Conclusions The concurrent administration of ketoconazole and donepezil produces no change in ketoconazole plasma concentrations, but a statistically significant change in donepezil plasma concentrations. These observed changes, which are smaller than those produced by ketoconazole for other agents sharing the CYP-3A4 pathway, are most likely the result of donepezil also being metabolized by CYP-2D6, as well as its slow rate of clearance from plasma.

Keywords: donepezil, ketoconazole, drug–drug interaction, acetylcholinesterase inhibitor

Introduction
Cholinergic deficit is one of the major pathological features of Alzheimer’s disease. This deficit has been associated with the loss of cognition and memory, the primary symptoms of this disorder [1]. In an attempt to alleviate these clinical symptoms, therapeutic investigations have focused on enhancing the action of the remaining cholinergic neurones. To date, the most successful approach has been in the development of cholinergic agents, in particular, acetylcholinesterase (AChE) inhibitors [2, 3]. The first of the ‘new-generation’ AChE inhibitors used in the symptomatic treatment of mild–moderate Alzheimer’s disease is donepezil HCl.

Donepezil HCl (also known as E2020 or Aricept\(^\text{®}\)), the registered trademark of Eisai Co. Ltd, Tokyo, Japan, is a distinct, pipedine-based agent that inhibits the enzyme AChE [4–8]. In pre-clinical investigations, donepezil was found to have a greater specificity for AChE as opposed to butyrylcholinesterase (BuChE) than either physostigmine or tacrine, and a longer duration of action than either of these drugs [9]. In clinical trials, treatment with single daily doses of either 5 or 10 mg of donepezil significantly improved cognition and global function in patients with Alzheimer’s disease [10–12]. Treatment with donepezil was well tolerated and was not associated with hepatotoxicity. Adverse events, when present, tended to be mild and transient gastrointestinal disturbances, such as abdominal pain, nausea and vomiting. All of these events are consistent with an increase in cholinergic stimulation and resolved within a few days for most patients, without the need for dose modification [11].

In healthy individuals, the pharmacokinetics of donepezil are characterized by hepatic metabolism and slow plasma clearance (0.131 h\(^{-1}\) kg\(^{-1}\)) [13]. Owing to its long half-life (approximately 70 h), donepezil is therapeutically effective using once-daily dosing.
Ketoconazole is an imidazole derivative that is administered as a treatment for superficial and systemic fungal infections [14]. It is a specific, potent inhibitor of the cytochrome P-450 isoenzyme CYP-3A4 via which numerous compounds are metabolized. It has been shown to reduce the metabolism of other drugs sharing the CYP-3A4 pathway, including cyclosporin, warfarin, terfenadine, astemizole and some benzodiazepines [15, 16]. In addition, drug interactions with ketoconazole have also been reported for other medications, including corticosteroids, antihistamines and antacids [17–19].

As donepezil is predominantly metabolized by CYP-3A4, and to a lesser extent by CYP-2E1 (Aricept® US package insert, 1998), concurrent administration of ketoconazole might affect plasma concentrations of donepezil. Therefore, the primary objective of this study was to examine the pharmacokinetics of donepezil and ketoconazole administered separately and in combination, following single and multiple oral doses.

Methods

Subjects

Entry into the study was confined to healthy, non-smoking volunteers between 18 and 45 years of age who were within 20% of ideal body weight based on the Metropolitan Insurance Company Height and Weight Tables (1983). Subjects with evidence of clinically significant hepatic, gastrointestinal, renal, respiratory, endocrine, haematological, neurological, psychiatric or cardiovascular system abnormalities were specifically excluded from the study, as were those who had a known or suspected history of alcohol or drug misuse or a positive urine drug screen. None of the subjects had donated blood or had received investigational or prescription medications within 1 month of commencing trial medication.

The study was conducted in accordance with the principles stated in the Declaration of Helsinki, and the Institutional Review Board for Investigations Involving Human Subjects, Harris Laboratories, Lincoln, Nebraska, USA, approved the protocol. All subjects gave written informed consent prior to participation in the study.

Protocol

This was an open-label, randomized, multiple-dose, three-period crossover study. The three randomized treatments administered in this study were (1) donepezil HCl, 5 mg tablet, (2) ketoconazole, 200 mg tablet (Nizoral®, Janssen), and (3) donepezil, 5 mg + ketoconazole, 200 mg. Each treatment period was 7 days in duration and was followed by a 3-week, drug-free washout period. The dose of donepezil was chosen on the basis of results of clinical efficacy studies conducted in the USA, and the dose of ketoconazole is the recommended therapeutic dose for treatment provided by the Physicians’ Desk Reference.

All volunteers were screened by medical history, ECG and laboratory and physical examinations ≤2 weeks prior to the start of the study. For each treatment period, subjects were admitted to the study site on the evening of day 0, at least 12 h prior to drug administration. Subjects were fasted overnight (8 h) prior to receiving their first dose of medication on the morning of day 1. Following drug administration, blood samples for analytical determinations were collected at specified intervals during the next 24 h. Subjects were discharged from the study site the following morning, after providing a 24-h blood sample.

The subjects returned to the clinic as out-patients for the next four mornings (48, 72, 96 and 120 h) to provide trough blood samples for pharmacokinetic analysis and to receive their daily dose of medication. They returned to the clinic as out-patients for the next six mornings to provide blood for post-dose pharmacokinetic analyses (to 168 h). During the course of the treatment period, subjects were not allowed to consume caffeine-containing food or drinks, and physical exercise was limited to normal walking.

Sample collection and analysis

Venous blood samples for the determination of donepezil and/or ketoconazole concentrations in plasma were collected 1 h prior to drug administration, and at 5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 48, 72, 96 and 120 h post-dose on days 1 and 7. Additional samples were taken at 144 and 168 h after the last dose of medication on day 7 of each treatment period.

Immediately after collection, the blood samples were placed on ice and centrifuged for 15 min (2000 × g at 4 °C). Plasma was then removed and transferred into polypropylene tubes, which were stored upright at −20 °C until analysis. Plasma concentrations of donepezil (hydrochloride salt) were determined using a specific high-performance liquid chromatography (HPLC) method with UV detection [20]. Ketoconazole was also analysed using a standard HPLC method with UV detection. The limits of detection for these assays were 2 ng ml−1 for donepezil and 0.04 ng ml−1 for ketoconazole.

Pharmacokinetic assessments

Characterization of donepezil and/or ketoconazole pharmacokinetics for each treatment phase was performed by analysing blood samples collected over a 24-h period following initial dose administration, and a 168-h period following final dose administration.

Pharmacokinetic parameters for both drugs were estimated by a non-compartmental method. Peak plasma concentration (Cmax) and the time at which it occurred (tmax) were recorded from the observed values. The terminal disposition phases of both donepezil and ketoconazole were identified by visual inspection of each subject’s log concentration–time curve. The terminal disposition rate constant (t1/2) was calculated as ln 2/λz, and the area under the plasma concentration–time curve from 0 to 24 h (AUC[0–24]) was estimated using the trapezoidal rule. The
accumulation ratio (Ra) was defined as AUC$_{0-24}$ on day 7 divided by AUC$_{0-24}$ on day 1.

### Statistical analysis

Pharmacokinetic parameters for all three treatment groups were calculated following single-dose administration on day 1 and the final dose on day 7. An analysis of variance model (ANOVA), accounting for the effects of treatment, period, sequence and subject, was used to compare these parameters between days and between treatment periods. The type III sum of squares for all model effects was used to determine statistical significance at the 0.05 level.

### Results

#### Subjects

A total of 21 subjects were enrolled into the trial and 18 successfully completed all three treatment phases. They ranged in age from 20 to 45 years (mean 31.2 years), in height from 158 to 189 cm (mean 174.1 cm), and in body weight from 54.5 to 81.5 kg (mean 72.1 kg). Of the 21 volunteers, 19 were Caucasian, one was Hispanic and one was Asian.

#### Pharmacokinetics of donepezil

Mean plasma donepezil concentrations were calculated for each time-point for both donepezil treatment groups (donepezil alone, donepezil + ketoconazole). A plasma concentration–time plot of these data is presented in Figure 1. On day 1, a statistically significant difference was observed between the donepezil and the donepezil + ketoconazole treatment groups in terms of C$_{max}$ (P=0.01) and AUC$_{0-24}$ of donepezil (P=0.001). The combination group had a 12% greater C$_{max}$ (9.5 ng ml$^{-1}$ versus 8.4 ng ml$^{-1}$) and a 12% greater AUC$_{0-24}$ (135.2 ng h ml$^{-1}$ versus 118.7 ng h ml$^{-1}$) compared with the donepezil-only group (Table 1). No significant difference in t$_{max}$ was observed between the groups. No significant sequence effects were observed.

On day 7, a statistically significant difference was again observed between the donepezil and the donepezil + ketoconazole groups in terms of C$_{max}$ and AUC$_{0-24}$ of donepezil (P<0.0001 for both). As shown in Table 2, the donepezil + ketoconazole group had a 26.8% greater C$_{max}$ (37.7 ng ml$^{-1}$ versus 27.6 ng ml$^{-1}$) and a 26.3% greater AUC$_{0-24}$ (680.9 ng h ml$^{-1}$ versus 501.1 ng h ml$^{-1}$) than the donepezil-only group. There was also a significant (P<0.0001) increase of 16% in the rate of donepezil accumulation (Ra) for the combination group, compared with the donepezil-only group (Table 2). Although this increase is not considered to be clinically relevant. No significant sequence effects were observed.

#### Study state pharmacokinetics

As this study involved only 7 consecutive days of drug administration during each treatment period and donepezil steady-state concentrations are not attained until 14–21 days, the effect of concomitant administration at steady state was estimated using pharmacokinetic modelling. Projected steady-state plasma concentrations of donepezil during concomitant administration with ketoconazole were calculated by fitting day 1 and day 7 data to both a one-compartment model and a sigmoidal E$_{max}$ model. The mean trough concentration of donepezil at steady state was estimated to be 30.7 ng ml$^{-1}$ and 27.7 ng ml$^{-1}$ for the two models, respectively. Previous phase I studies with donepezil have calculated C$_{max}$ to be 21.4 ng ml$^{-1}$ [21]. Using the one-compartment model, these data suggest that the concurrent administration of ketoconazole will produce a 30% increase in donepezil concentrations at steady state. Using the E$_{max}$ model, a 23% increase in donepezil concentrations at steady state is predicted.

#### Pharmacokinetics of ketoconazole

Mean plasma ketoconazole concentrations were calculated per time-point for each ketoconazole treatment group.

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**Table 1**: Comparison of donepezil pharmacokinetic parameters on day 1 (mean±SE).

<table>
<thead>
<tr>
<th></th>
<th>Donepezil</th>
<th>Donepezil + ketoconazole</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{max}$ (ng ml$^{-1}$)</td>
<td>8.4±0.4</td>
<td>9.5±0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>t$_{max}$ (h)</td>
<td>3.8±0.2</td>
<td>3.6±0.3</td>
<td>0.50</td>
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<tr>
<td>AUC$_{0-24}$ (ng h ml$^{-1}$)</td>
<td>187.2±5.3</td>
<td>135.2±7.5</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Table 2**: Comparison of donepezil pharmacokinetic parameters on day 7 (mean±SE).

<table>
<thead>
<tr>
<th></th>
<th>Donepezil</th>
<th>Donepezil + ketoconazole</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{max}$ (ng ml$^{-1}$)</td>
<td>27.6±1.7</td>
<td>37.7±2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>t$_{max}$ (h)</td>
<td>4.2±0.2</td>
<td>3.9±0.2</td>
<td>0.32</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (ng h ml$^{-1}$)</td>
<td>501.1±38.3</td>
<td>680.9±48.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>t$_{1/2}$ (h)</td>
<td>58.4±2.1</td>
<td>60.0±3.2</td>
<td>0.46</td>
</tr>
<tr>
<td>Ra</td>
<td>4.2±0.2</td>
<td>5.0±0.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
(ketoconazole alone, donepezil + ketoconazole). A plasma concentration–time plot of this mean data is presented in Figure 2. Results of the ketoconazole pharmacokinetic analyses are summarized in Tables 3 and 4. On days 1 and 7, no statistically significant differences in ketoconazole pharmacokinetics were observed when ketoconazole administered alone was compared with ketoconazole administered in combination with donepezil. No significant sequence effects were observed.

Safely

All treatments were well tolerated and no serious or unexpected adverse events occurred during the course of the study. Treatment-emergent signs and symptoms were mild or moderate in severity, and the incidence and severity of symptoms for donepezil and ketoconazole administered in combination were similar to those for donepezil administered alone.

Discussion

Ketoconazole is a potent inhibitor of the P-450 isoenzyme CYP-3A4. Several reports have demonstrated that concurrent administration of this drug can reduce the metabolism of other drugs [15–19]. As CYP-3A4 is the primary route of donepezil metabolism, it is important to investigate whether co-administration with ketoconazole would produce any changes in donepezil metabolism and/or plasma concentrations.

The concurrent administration of both single and multiple doses of donepezil (5 mg) + ketoconazole (200 mg) resulted in a significant increase in the plasma level of donepezil compared with the single- and multiple-dose administration of donepezil alone. This change in donepezil plasma concentration was reflected by increases in C_{max} and AUC_{0–24} on both day 1 and day 7. There was no difference in the t_{1/2} values of the two groups on either day 1 or 7 and no difference in t_{1/2} on day 7. The rate of donepezil accumulation in plasma (as reflected by the accumulation ratio, R_{A}) was 16% higher for the donepezil + ketoconazole group after 7 days of drug treatment. Clinical studies have shown that donepezil accumulation continues until steady state is achieved within 14–21 days [10]. As each treatment lasted for 7 days, steady-state donepezil plasma concentrations during concurrent administration of both drugs were estimated using both a sigmoidal E_{max} model and a one-compartment model. Donepezil concentrations were projected to increase by 23% in the E_{max} model and by 30% in the one-compartment model.

Although both the observed and estimated increases in donepezil concentrations were significant, they are smaller than those produced by ketoconazole administered concomitantly with other agents sharing the CYP-3A4 pathway. This is most probably the result of donepezil also being metabolized by CYP-2D6, as well as its slow rate of clearance from plasma.

Conversely, ketoconazole pharmacokinetics were not affected by the concurrent administration of donepezil. The lack of effect on ketoconazole plasma concentrations was expected, and is consistent with the results obtained from in vitro studies with donepezil (Aricept® US package insert, 1998). Isoform-selective substrate studies conducted in human hepatic microsomes determined that the donepezil concentrations required for 50% inhibition (IC_{50}) of P-450 isoenzymes 1A2, 2C9, 2C19, 2D6 and 3A4 were all greater than 100 μM. In addition, the mean k_{i} values for CYP-3A4 and CYP-2D6 were found to be 131 μM and 47 μM, respectively. Clinical studies have estimated that the steady-state concentration for the 10 mg dose of donepezil is approximately 164 nM at the level of the hepatocyte. As it is expected that therapeutic concentrations of donepezil are more than 280-fold lower than the lowest k_{i} obtained for CYP-2D6 and almost 800-fold lower than the k_{i} observed with CYP-3A4, it is anticipated that donepezil will not inhibit the metabolism of other drugs metabolized via these P-450 isoenzymes.

In summary, the concurrent administration of donepezil
and ketoconazole was found to produce no change in donepezil plasma concentrations, but did result in an increase in donepezil plasma concentrations estimated to be 23–30% at steady state. The results of this study support in vitro findings (unpublished data) demonstrating that donepezil HCl is primarily metabolized by both CYP-3A4 and CYP-2D6, and that drugs that inhibit the CYP-3A4 pathway will also inhibit donepezil metabolism to some degree. However, it is unclear whether the metabolism of the drug is altered in such a way as to produce a changed pattern of metabolites. As the metabolites of donepezil are essentially clinically inactive (due to both low plasma concentrations as well as an inability to cross the blood–brain barrier), it is unlikely that even a substantial change in the metabolic processing of the drug would result in either a modification of drug effect or an increase in adverse events. Moreover, both the observed and the estimated increases in donepezil plasma concentrations are smaller than those produced by ketoconazole for other agents sharing the CYP-3A4 pathway and are thus unlikely to be clinically relevant. These findings suggest that dose modifications should not be required in patients to whom ketoconazole or another CYP-3A4 inhibitor are administered concurrently with donepezil.

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References