Short Communication

A study of the interactions between donepezil and yokukansan, cimetidine, and ketoconazole in rats

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Abstract

Donepezil, a drug used for the treatment of Alzheimer's disease, is metabolized mainly by CYP3A4 and CYP2D6 in humans, and its plasma concentration is elevated by coadministration of ketoconazole or cimetidine. Because yokukansan is reported to improve behavioral and psychological symptoms of dementia in Alzheimer's disease, it is likely that yokukansan is prescribed in combination with donepezil. We investigated whether ketoconazole, cimetidine and yokukansan affects the disposition of donepezil in rats.

Ketoconazole (10 mg/kg) or cimetidine (200 mg/kg) was administered intraperitoneally to male Wistar rats. Donepezil hydrochloride (5 mg/kg) was then administered orally, and plasma donepezil concentration was measured by HPLC-UV. The plasma levels of donepezil were higher in the ketoconazole-coadministered group and the cimetidine-coadministered group than in the control group. The area under the plasma concentration-time curve were also higher in these two groups than in the control group. In contrast, after repeated oral administration of yokukansan extract powder (1 g/kg) once a day for 7 days, plasma donepezil levels following oral administration of donepezil hydrochloride (5 mg/kg) did not differ from those in the control group. These results indicate that ketoconazole or cimetidine coadministered with donepezil elevates plasma donepezil levels in rats, but coadministered yokukansan has no effect on the disposition of donepezil. These results suggest that yokukansan is unlikely to cause pharmacokinetic interactions when coadministered with donepezil in clinical practice.

Key words donepezil, yokukansan, ketoconazole, cimetidine, drug interaction.

Abbreviations AUC, area under the plasma concentration-time curve; BPSD, behavioral and psychological symptoms of dementia; Cmax, maximum plasma concentration; CYP, cytochrome P450; PEG, polyethylene glycol; SE, standard error; Tmax, time-to-maximum plasma concentration.

Introduction

In our investigations of possible drug interactions between Western medicines and traditional Japanese medicines, we have focused on drug-metabolizing enzymes and transporters, which play important roles in drug interactions, and we have studied the effects of traditional Japanese medicines on the activities of enzymes/transporters. Both in vitro and in vivo studies show that yokukansan has little inhibitory effect on cytochrome P450 (CYP), a primary drug-metabolizing enzyme, and P-glycoprotein, a transporter involved in the absorption, distribution, and excretion of many
Yokukansan has been used to control such conditions as nervousness, and recently also reported to improve behavioral and psychological symptoms of dementia (BPSD) of Alzheimer’s disease. At present, donepezil hydrochloride is the only drug covered by the National Health Insurance for the symptomatic treatment of Alzheimer’s disease in Japan, and it is likely that yokukansan is used in combination with donepezil. To investigate whether yokukansan affects the disposition of donepezil, we coadministered yokukansan and donepezil orally to rats.

Donepezil is eliminated from the body mainly by hepatic metabolism, and the urinary recovery of unchanged donepezil is low in both humans and rats. Metabolic studies using human liver microsomes have reported that donepezil is metabolized by CYP3A4 to produce N-dealkylate (M4) and by CYP2D6 to produce O-demethylates (M1 and M2), followed by glucuronidation of M1 and M2. Although the enzymes responsible for donepezil metabolism have not been identified in rats, the main metabolites are N-dealkylate (M4), O-demethylates (M1 and M2), and their glucuronides, suggesting that humans and rats have similar metabolic pathways for donepezil (Fig. 1).

Fig. 1 The proposed metabolic pathways of donepezil in rats (Matsui et al., 2000).
actions result from the inhibition of CYP-mediated donepezil metabolism by these drugs. We investigated the effects of ketoconazole and cimetidine on donepezil disposition in rats to evaluate the potential for these drugs to be used as positive controls for donepezil interaction studies. The doses were determined based on reported drug interaction studies involving the respective drugs.\textsuperscript{11,12}

**Materials and Methods**

**Reagents:** Donepezil hydrochloride and its metabolites (M1, M2, M4, and M9) and yokukansan extract powder were provided by Tsumura & Co. (Tokyo, Japan). Ketoconazole, cimetidine, polyethylene glycol (PEG) 400, sodium chloride, 2-propanol, hexane, acetonitrile, ammonium acetate, acetic acid, sodium tetraborate decahydrate, and sodium hydroxide were obtained from Wako Pure Chemicals, Ltd. (Osaka, Japan). Ethyl ether was obtained from Showa Ether Co. (Tokyo, Japan). For the other reagents used in this study, those with the highest grade available commercially were purchased.

**Animals:** Male Wistar rats were obtained from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan). The animals were housed under the conditions of 24 ± 1°C and 55 ± 5% humidity with a light-dark cycle of 8:00-20:00 h. The animals had free access to food (solid feed for experimental animals MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water.

The experiments were carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University.

**Effects of ketoconazole and cimetidine on the disposition of donepezil:** Thirty minutes after 4 mL/kg of either ketoconazole (2.5 mg/mL), cimetidine (50 mg/mL), or vehicle (PEG 400:saline, 1:1, v/v) was administered intraperitoneally to 7-week-old male Wistar rats, 5 mL/kg of donepezil hydrochloride (1 mg/mL) dissolved in water was administered orally. The doses of ketoconazole, cimetidine, and donepezil hydrochloride were 10, 200, and 5 mg/kg, respectively. At 30 minutes and 1, 2, 4, and 8 hours after administration of donepezil hydrochloride, rats were anesthetized with ethyl ether and blood was collected from the orbital venous plexus. The blood samples were centrifuged at 860 × g for 15 minutes at 4°C, and plasma was separated and stored at -80°C until measurement of donepezil concentration.

**Effects of yokukansan on the disposition of donepezil:** Yokukansan suspended in water (200 mg/mL; 5 mL/kg) was administered orally to 6-week-old male Wistar rats once a day for 7 days. As a control, 5 mL/kg of water was administered orally once a day for 7 days. On the 8th day, 5 mL/kg of donepezil hydrochloride (1 mg/mL) was administered orally; the doses of yokukansan and donepezil hydrochloride were 1 g/kg and 5 mg/kg, respectively. Blood samples were collected from each rat, and the plasma was isolated and stored as mentioned above.

**Measurement of donepezil concentration:** Two hundred microliters of 50 mM borate buffer (pH 10) and 1 mL of a mixture of 5% isopropanol and hexane were added to 200 μL of plasma and centrifuged at 1,000 × g for 10 minutes at 10°C. Eight hundred microliters of the resulting supernatant (organic layer) was evaporated to dryness under a gentle stream of nitrogen gas, 30 μL of the mobile phase was added to the residues and mixed by vortex mixer for 1 minute, and 20 μL was injected into an HPLC system.

The HPLC system comprised a 600S Controller (Waters Japan, Tokyo, Japan), 616 Pump (Waters Japan), and 717 plus Autosampler (Waters Japan). The analytical column was an Inertsil C\textsubscript{18} ODS-3 (4.6 mm × 250 mm) of average 5-μm particle size (GL Sciences Inc., Tokyo, Japan). For the mobile phase, 10 mM of acetic acid buffer (pH 5.0) and acetonitrile, 25: 75 (v/v), was used for studies of ketoconazole and cimetidine, and 10 mM acetic acid buffer (pH 5.0) and acetonitrile, 5:95 (v/v), was used for studies of yokukansan. Donepezil concentration was measured at a flow rate of 1.0 mL/min at 40°C at 315 nm using a UV spectrophotometer.\textsuperscript{13}

**Data analysis:** All tests were carried out using 3 to 5 rats for each group, and the values are presented as mean ± standard error (SE). The area under the plasma concentration-time curve (AUC) of donepezil was
calculated for each rat up to the final sampling point (8 hours) using the trapezoidal rule. The elimination rate constant (kₑ) and elimination half-life (t₁/₂=0.693/kₑ) were calculated from the concentration profiles of the elimination phase. The maximum plasma concentration (Cmax) and time-to-maximum plasma concentration (Tmax) were obtained from actual measurements. Student’s t test was used to examine the significance of the differences and a p value of less than 0.05 was considered significant.

Results

Effects of ketoconazole and cimetidine on the disposition of donepezil: The plasma concentration profiles of donepezil are shown in Fig. 2. Plasma concentrations of donepezil were higher at each time in the ketoconazole coadministration group than in the control group, whereas the concentrations were higher in the cimetidine coadministration group than in the control group at 2, 4, and 8 hours after administration. As shown in Table 1,

![Figure 2](image2.png)

**Fig. 2** Effects of ketoconazole or cimetidine administration on plasma concentrations of donepezil in rats. Thirty minutes after intraperitoneal administration of ketoconazole (10 mg/kg, □), cimetidine (200 mg/kg, △) or solvent (◆), donepezil hydrochloride (5 mg/kg) was administered orally. The values are expressed as mean ± SE, n=3.

![Figure 3](image3.png)

**Fig. 3** Effects of yokukansan administration on plasma concentrations of donepezil in rats. After oral administration of either yokukansan (1 g/kg, [ ]) or water ([ ] ) once a day for 7 days, donepezil hydrochloride (5 mg/kg) was administered orally. The values are expressed as mean ± SE, n=4-5.

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<tr>
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<th>AUC₉₈,₈ (ng·h/mL)</th>
<th>kₑ (h⁻¹)</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (h)</th>
<th>T₁/₂ (h)</th>
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<tbody>
<tr>
<td>control</td>
<td>121 ± 19</td>
<td>0.134 ± 0.030</td>
<td>23.6 ± 5.6</td>
<td>0.83 ± 0.14</td>
<td>5.84 ± 1.03</td>
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<td>ketoconazole (10 mg/kg, i.p.)</td>
<td>196 ± 20</td>
<td>0.106 ± 0.024</td>
<td>36.7 ± 4.2</td>
<td>1.17 ± 0.36</td>
<td>8.13 ± 2.36</td>
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<td>cimetidine (200 mg/kg, i.p.)</td>
<td>209 ± 60</td>
<td>0.137 ± 0.029</td>
<td>33.1 ± 8.1</td>
<td>2.17 ± 0.83</td>
<td>6.09 ± 1.68</td>
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<td>mean ± SE, n=3</td>
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<th>AUC₉₈,₈ (ng·h/mL)</th>
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<th>Tmax (h)</th>
<th>T₁/₂ (h)</th>
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<tr>
<td>control</td>
<td>192 ± 32</td>
<td>0.151 ± 0.029</td>
<td>52.9 ± 7.4</td>
<td>0.50 ± 0.00</td>
<td>5.99 ± 3.71</td>
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<td>yokukansan (1 g/kg, p.o.)</td>
<td>201 ± 12</td>
<td>0.172 ± 0.011</td>
<td>51.6 ± 1.8</td>
<td>0.60 ± 0.09</td>
<td>4.10 ± 0.26</td>
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<td>mean ± SE, n=4-5</td>
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Table 1  Effects of ketoconazole or cimetidine coadministration on pharmacokinetic parameters of donepezil after oral administration of donepezil hydrochloride (5 mg/kg) in rats.

Table 2  Effects of yokukansan coadministration on pharmacokinetic parameters of donepezil after oral administration of donepezil hydrochloride (5 mg/kg) in rats.
both the AUC up to 8 hours after administration and Cmax value were higher in the ketoconazole coadministration group and cimetidine coadministration group than in the control group, although these differences were not significant. Tmax was prolonged only in the cimetidine coadministration group compared with the control group, although this difference was not significant (Table 1).

**Effects of yokukansan on the disposition of donepezil:**
Plasma donepezil concentrations did not differ significantly between the yokukansan coadministration group and the control group (Fig. 3). The AUC, Cmax, and other pharmacokinetic parameters were also similar for both groups (Table 2).

**Discussion**

This study investigated the effects of ketoconazole, cimetidine and yokukansan on the disposition of donepezil, a drug for the treatment of Alzheimer’s disease using in vivo experiments in rats.

Both the AUC and Cmax values of plasma donepezil were higher when donepezil hydrochloride (5 mg/kg) was administered orally 30 minutes after intraperitoneal administration of ketoconazole (10 mg/kg) or cimetidine (200 mg/kg) compared with the values after administration of donepezil hydrochloride alone (Fig. 2, Table 1). These results are likely to have resulted from the inhibition of donepezil metabolism by ketoconazole or cimetidine. Although these two drugs, which have an imidazole ring in common, are regarded as inhibitors of CYP3A4 through the same mechanism, the Tmax of donepezil was prolonged only after cimetidine coadministration in the present study. It has been suggested that donepezil is a substrate of mouse P-glycoprotein.\(^{14}\) Ketoconazole inhibits human and mouse P-glycoprotein,\(^{15-17}\) whereas cimetidine has a weak inhibitory effect on P-glycoprotein.\(^{16}\) Such differences might have caused the different effects of these drugs on the disposition of donepezil observed in this study. In contrast, plasma donepezil concentration in rats that received oral administration of donepezil hydrochloride (5 mg/kg) after oral administration of yokukansan (1 g/kg) once a day for 7 days did not differ from the concentration in the control rats that received donepezil hydrochloride alone (Fig. 3). These results are consistent with the previous findings that yokukansan has little inhibitory effect on CYP isozymes and P-glycoprotein\(^{12}\) and suggest that repeated administration of yokukansan does not affect the disposition of donepezil.

In summary, this study has shown that ketoconazole or cimetidine administration increases plasma donepezil concentration in rats, whereas yokukansan does not affect the disposition of donepezil. Although the possibility of species differences in the enzymes responsible for donepezil metabolism should be taken into consideration, these results suggest that yokukansan is unlikely to cause pharmacokinetic interactions when coadministered with donepezil in clinical practice.

**References**

6) Matsui, K., Taniguchi, S. and Yoshimura, T.: Identification of cytochrome P450 involved in the metabolism of


