Effects of Serotonin-3 Receptor Antagonists on Cytochrome P450 Activities in Human Liver Microsomes

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The effects of three serotonin-3 (5-HT3) receptor antagonists, azasetron, ondansetron, and ramosetron, on cytochrome P450 (CYP) 1A2-mediated 7-ethoxyresorufin O-deethylation, CYP2C9-mediated tolbutamide hydroxylation, CYP2C19-mediated S-mephenytoin 4′-hydroxylation, CYP2D6-mediated debrisoquine 4-hydroxylation, CYP2E1-mediated chlorzoxazone 6-hydroxylation, CYP3A4-mediated nifedipine oxidation, and CYP3A4-mediated testosterone 6β-hydroxylation activities in human liver microsomes were compared. Azasetron and ramosetron at a concentration of 1 or 10 μM neither inhibited nor stimulated any of the metabolic activities. On the other hand, ondansetron competitively inhibited CYP1A2 and CYP2D6 activities, and the inhibition constants (KI) were 3.2 and 21.0 μM, respectively, which are much higher than the reported plasma concentrations after clinical intravenous or oral dosing. The free fractions of the three 5-HT3 receptor antagonists in the incubation mixture estimated by ultracentrifugation were more than 68.6%. These results suggest that azasetron, ondansetron, and ramosetron do not cause clinically significant interactions with other drugs that are metabolized by CYPs via the inhibition of metabolism.

Key words serotonin-3 receptor antagonist; azasetron; ondansetron; ramosetron; cytochrome P450; human liver microsome

Cytochrome P450s (CYPs) comprise a superfamily of enzymes which catalyze the oxidation of a wide variety of xenobiotic chemicals, including drugs and carcinogens.1–3) Multiple-drug therapy is a common therapeutic practice, particularly in patients with several diseases or conditions, and many drug–drug interactions involving metabolic inhibition have been reported.4,5)

The serotonin-3 (5-HT3) receptor antagonists ramosetron, azasetron, and ondansetron are widely used in the treatment of nausea and emesis induced by cytotoxic chemotherapy and radiotherapy.6) Therefore 5-HT3 receptor antagonists are coadministered with drugs including anticancer drugs in most cases, and the possibility of interactions between them and other drugs exists. Many anticancer drugs, such as cyclophosphamide, tamoxifen, and paclitaxel, are metabolized in many cases, and the possibility of interactions between them and other drugs exists. Many anticancer drugs, such as cyclophosphamide, tamoxifen, and paclitaxel, are metabolized...
6-hydroxylation, nifedipine oxidation, and testosterone 6β-hydroxylation by human liver microsomes were 0.22, 150.8, 27.3, 83.9, 47.7, 12.2, and 50.3 μM, respectively,\(^8,9\) concentrations of 7-ethoxyresorufin, tolbutamide, S-mephenytoin, debrisoquine, chlorzoxazone, nifedipine, and testosterone were 0.25, 200, 30, 100, 50, 10, and 50 μM, respectively, which are around the expected \(K_m\) values. Incubation was carried out at 37 °C for 5 min (for testosterone 6β-hydroxylation), 10 min (for 7-ethoxyresorufin O-deethylation, chlorzoxazone 6-hydroxylation, and nifedipine oxidation), 30 min (for tolbutamide hydroxylation and S-mephenytoin 4'-hydroxylation), or 60 min (for debrisoquine 4-hydroxylation).\(^8,9\) In preliminary experiments, the linearity of the reaction with incubation time and protein concentration was confirmed for each assay condition.

**Determination of Free Fraction in the Incubation Mixture** The incubation mixture consisted of human liver microsomes (0.05—0.5 mg/ml), 1, 10 or 100 μM 5-HT₃ receptor antagonists, and 100 mM phosphate buffer (pH 7.4) in a final volume of 500 μl. After 5-min incubation at 37 °C, the mixture was centrifuged at 100000 \(g\) for 60 min at 37 °C\(^{10—12}\), and the concentration of 5-HT₃ receptor antagonists in the supernatant was measured on HPLC with an analytical column Inertsil ODS-3 (150×4.6 mm i.d., GL Sciences, Tokyo, Japan) equipped with a TSK-guardgel ODS-80Ts (3.2×15 mm, Tosoh, Tokyo, Japan). The column temperature was set at 40 °C. The elution was conducted with 25% acetonitrile in 20 mM perchlorate buffer (pH 2.5) at a flow rate of 1 ml/min. The elution of the analytes was monitored with UV detection (305 nm for ondansetron and 311 nm for ramosetron), and azasetron was determined fluorometrically (excitation wavelength 318 nm and emission wavelength 380 nm).

**Data Analysis** All data were analyzed using the average of duplicate or triplicate determinations, and the inhibition constant \(K_i\) was estimated by fitting the inhibition curves to Eq. 1 or Eq. 2 when the inhibition type was competitive or noncompetitive, respectively:

\[
v = V_{max} \cdot \frac{S}{K_m(1+I/K_i)+S} \quad (1)
\]

\[
v = \frac{V_{max}(1+I/K_i) \cdot S}{(K_m+S)} \quad (2)
\]

where \(v\), \(S\), \(I\), \(V_{max}\), and \(K_m\) are the velocity of the metabolite formation and the concentrations of substrate and inhibitor, the maximum velocity of the metabolite formation, and the apparent Michaelis–Menten constant, respectively. These equations were fitted to data by means of a computer program (MULTI),\(^{13}\) and fitting evaluation was carried out using Akaike’s information criterion.\(^{14}\)

**RESULTS**

**Inhibition of CYP Activities by 5-HT₃ Receptor Antagonists** The inhibitory effects of azasetron, ondansetron, and ramosetron on CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 (NF) activities in human liver microsomes were shown in Fig. 1. The three 5-HT₃ receptor antagonists neither inhibited nor stimulated any of the metabolic activities except that ondansetron at a concentration of 10 μM inhibited CYP1A2 and CYP2D6 activities by 44% and 23%, respectively. Therefore the effects of ondansetron on CYP1A2 and CYP2D6 activities were investigated in detail (Fig. 2). Ondansetron competitively inhibited CYP1A2-mediated 7-ethoxyresorufin O-deethylation and CYP2D6-mediated debrisoquine 4-hydroxylation, with \(K_i\) values of 3.2 and 21.0 μM, respectively.
Free Fraction in the Incubation Mixture  

The free fractions of 5-HT₃ receptor antagonists in the incubation mixture (0.05—0.5 mg protein/ml) were determined at 1, 10, and 100 (ondansetron) μM after ultracentrifugation (Table 1). The free fractions of azasetron, ondansetron, and ramosetron were 81.4—96.7%, 79.7—98.5%, and 68.6—92.1%, respectively. Although a minor decrease in free fraction at the higher protein concentration was observed for ondansetron and ramosetron, the independence of compound concentration was shown for the three 5-HT₃ receptor antagonists as well as many other drugs including imipramine, propranolol, troglitazone, and amitriptyline.¹⁵—¹⁸)

DISCUSSION

After oral or intravenous administration in humans, 48—67% of azasetron is excreted as an unchanged drug in urine,¹⁹—²²) and there are few reports on the metabolic enzymes of azasetron. On the other hand, the metabolism of ondansetron is catalyzed by multiple CYP isoforms, including the CYP1A2, CYP2D6, and CYP3A subfamily,²³) and ramosetron is metabolized by CYP1A1/2 and CYP2D6 (Astellas Pharma Inc., unpublished results). In this study, we found that azasetron, ondansetron, and ramosetron at a concentration of 1 or 10 μM neither inhibited nor stimulated any of the metabolic activities except that ondansetron competitively inhibited CYP1A2 and CYP2D6 activities with Ki values of 3.2 and 21.0 μM, respectively (Figs. 1, 2). In addition, the free fractions of three 5-HT₃ receptor antagonists in the incubation mixture estimated by ultracentrifugation were more than 68.6% (Table 1), suggesting that the protein binding in the incubation mixture is of minor importance. The maximum plasma concentrations (Cmax) after intravenous or oral dosing of azasetron, ondansetron, and ramosetron are

<table>
<thead>
<tr>
<th>Protein concentration in reaction mixture (mg/ml)</th>
<th>Azasetron (μM)</th>
<th>Free fraction (%)</th>
<th>Ondansetron (μM)</th>
<th>Free fraction (%)</th>
<th>Ramosetron (μM)</th>
<th>Free fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94.5 91.3</td>
<td>90.8 91.8</td>
<td>92.1 86.1</td>
<td>85.4 79.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>89.4 91.8</td>
<td>89.3 93.5</td>
<td>90.3 90.3</td>
<td>86.1 85.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>96.7 98.5</td>
<td>96.7 90.6</td>
<td>98.5 94.9</td>
<td>91.3 85.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>86.8 94.9</td>
<td>96.7 92.7</td>
<td>98.5 91.8</td>
<td>91.8 80.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>79.7 97.9</td>
<td>89.6 90.2</td>
<td>89.3 88.9</td>
<td>86.1 77.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>93.5 89.6</td>
<td>93.5 89.6</td>
<td>90.8 91.3</td>
<td>91.3 69.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Free Fractions of Azasetron, Ondansetron, and Ramosetron in Reaction Mixture Containing Human Liver Microsomes

<table>
<thead>
<tr>
<th>Dosing route³</th>
<th>Subject</th>
<th>Dose (mg)</th>
<th>Cmax or Cmax* (ng/ml, [μM])</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azasetron</td>
<td>Healthy male volunteers</td>
<td>2.5</td>
<td>65.8 [0.19]</td>
<td>Igarashi et al. (1992)¹⁹)</td>
</tr>
<tr>
<td></td>
<td>Healthy male volunteers</td>
<td>5</td>
<td>158.6 [0.45]</td>
<td>Igarashi et al. (1992)¹⁹)</td>
</tr>
<tr>
<td></td>
<td>Healthy male volunteers</td>
<td>10</td>
<td>190.5 [0.54]</td>
<td>Igarashi et al. (1992)¹⁹)</td>
</tr>
<tr>
<td></td>
<td>Healthy male volunteers</td>
<td>10</td>
<td>26.2—32.7 [0.075—0.093]</td>
<td>Hoshida et al. (1996)²⁰)</td>
</tr>
<tr>
<td></td>
<td>Healthy male volunteers</td>
<td>5</td>
<td>17.6—17.9 [0.050—0.051]</td>
<td>Hoshida et al. (1996)²⁰)</td>
</tr>
<tr>
<td></td>
<td>Young patients</td>
<td>10</td>
<td>25.9 [0.074]</td>
<td>Akasaka et al. (1996)²¹)</td>
</tr>
<tr>
<td></td>
<td>Elderly patients</td>
<td>10</td>
<td>33.3 [0.095]</td>
<td>Akasaka et al. (1996)²¹)</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>Healthy volunteers (CYP2D6 extensive metabolizers)</td>
<td>8</td>
<td>183 [0.62]</td>
<td>Ashforth et al. (1994)²⁴)</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers (CYP2D6 poor metabolizers)</td>
<td>8</td>
<td>199 [0.68]</td>
<td>Ashforth et al. (1994)²⁴)</td>
</tr>
<tr>
<td></td>
<td>Healthy young volunteers</td>
<td>8</td>
<td>83.4 [0.28]</td>
<td>Colthup et al. (1991)²⁷)</td>
</tr>
<tr>
<td></td>
<td>Healthy elderly volunteers</td>
<td>8</td>
<td>113.9 [0.39]</td>
<td>Colthup et al. (1991)²⁷)</td>
</tr>
<tr>
<td></td>
<td>Healthy young volunteers</td>
<td>8</td>
<td>26.4 [0.090]</td>
<td>Colthup et al. (1991)²⁷)</td>
</tr>
<tr>
<td></td>
<td>Healthy elderly volunteers</td>
<td>8</td>
<td>31.0 [0.11]</td>
<td>Colthup et al. (1991)²⁷)</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers (CYP2D6 extensive metabolizers)</td>
<td>4</td>
<td>10.90 [0.037]</td>
<td>Kumagaya et al. (1992)²⁶)</td>
</tr>
<tr>
<td></td>
<td>Healthy volunteers (CYP2D6 poor metabolizers)</td>
<td>8</td>
<td>23.82 [0.081]</td>
<td>Kumagaya et al. (1992)²⁶)</td>
</tr>
<tr>
<td></td>
<td>Healthy male volunteers</td>
<td>8</td>
<td>40.32 [0.14]</td>
<td>Kumagaya et al. (1992)²⁶)</td>
</tr>
<tr>
<td>Ramosetron</td>
<td>Healthy male volunteers</td>
<td>0.1</td>
<td>3.48 [0.012]</td>
<td>Nakajima et al. (1994)²⁷)</td>
</tr>
</tbody>
</table>

a) S, single dose; R, repeated dose.
summarized in Table 2.19–21,24–28 The clinical therapeutic doses of azasetron and ondansetron in Japan are 10 mg and 4 mg, respectively, for both intravenous and oral dosing, and those of ramosetron for intravenous and oral dosing are 0.3 mg and 0.1 mg, respectively. Therefore the clinical $C_{\text{max}}$ values of azasetron, ondansetron, and ramosetron after intravenous dosing, which were estimated by dose normalization of the reported $C_{\text{max}}$ in Table 2, are around 0.5 $\mu M$, 0.2–0.3 $\mu M$, and 0.04 $\mu M$, respectively, and 0.07–0.10 $\mu M$, 0.04–0.05 $\mu M$, and 0.001 $\mu M$, respectively, after oral dosing. No inhibition by azasetron and ramosetron at 10 $\mu M$ concentration, which is 20–250-fold, respectively, higher than the expected $C_{\text{max}}$ after intravenous dosing, was observed. Additionally, the $K_i$ values of ondansetron against CYP1A2 and CYP2D6 activities were 10–16- and 70–100-fold higher than the $C_{\text{max}}$ value of ondansetron after intravenous dosing.

The concentrations of azasetron, ondansetron, and ramosetron in the incubation mixture (10 $\mu M$) were 100–140-, 200–250-, and 10000-fold, respectively, higher than the expected $C_{\text{max}}$ values after clinical oral dosing. When the substrate concentration is much lower than the $K_i$ value, the ratio of intrinsic metabolic clearance ($CL_{in}$) in the presence and absence of the inhibitor can be expressed by the following equation, independent of the inhibition type, except in the case of uncompetitive inhibition:29,30

$$CL_{in|e} = \frac{CL_{in|e}^{(\text{inhibitor})}}{CL_{in|e}^{(\text{inhibitor})}} = \frac{1}{1 + I/R_i}$$

where $I$ is the unbound concentration of the inhibitor. Additionally, when the absorption rate is maximum, the maximum inflow blood concentration of the inhibitor into liver ($I_{in,max}$) after oral dosing can be expressed as

$$I_{in,max} = I_{in,max} - R_o + \{k_{in}/D/O_{in}\} F_o$$

where $I_{in,max}$, $R_o$, $D$, $O_{in}$, and $F_o$ represent the maximum plasma concentration of the inhibitor in the circulation, blood-to-plasma concentration ratio, absorption rate constant, dose, hepatic blood flow, and the fraction absorbed from the gastrointestinal tract into the portal vein, respectively. After oral dosing(s) of 10 mg azasetron, 4 mg ondansetron, or 0.1 mg ramosetron, the peak plasma concentrations ($I_{p,max}$) are expected to be 26–33 ng/ml (0.07–0.09 $\mu M$), 11–20 ng/ml (0.04–0.07 $\mu M$), or 0.34–0.47 ng/ml (0.001–0.002 $\mu M$), respectively.30,21,25–27 Free fractions of $I_{in,max}$ ($I_{p,max}$) for azasetron, ondansetron, and ramosetron after oral dosing are calculated using the free fraction in plasma ($f_p$) = 0.688, 0.12, and 0.09, respectively, with $R_o = 1$ ($R_0$ was assumed to be 1 because $R_0$ has not been reported.), $k_{in} = 0.1$ min$^{-1}$, $O_{in} = 1610$ ml/min, $F_o = 1$ to avoid false-negative predictions, to be 1.2 $\mu M$, 0.10–0.11 $\mu M$, and 0.002 $\mu M$, respectively. In this paper, we demonstrated that the inhibition of human CYPs by azasetron and ramosetron was not observed at 10 $\mu M$ (Table 1), which is 8–5000-fold higher than the predicted $I_{in,max}$. In addition, the 1+$I_{in,max}/K_i$ values of ondansetron for CYP1A2 and CYP2D6 were 1.03 and 1.005, respectively. Based on these estimations, as well as on the lack of inhibition of CYPs by azasetron and ramosetron, it is speculated that the three 5-HT3 receptor antagonists after intravenous or oral dosing would not cause clinically significant interactions with other drugs metabolized by CYPs via the inhibition of metabolism. There are no clinical reports that azasetron, ondansetron, and ramosetron increase the blood concentrations of other CYP-metabolized drugs as a result of inhibition of their metabolism.

Ondansetron competitively inhibited CYP1A2 and CYP2D6 activities with $K_i$ values of 3.2 and 21.0 $\mu M$, respectively. In addition, it appears that ondansetron inhibits the 4-hydroxylation of cyclophosphamide, an anticancer drug, with an IC$$_{50}$ value of >100 $\mu M$.31 Cyclophosphamide 4-hydroxylation is catalyzed by multiple CYP isoforms, with CYP2B6 displaying the highest activity,31 and multiple CYP forms, including the CYP1A2, CYP2D6, and CYP3A subfamily, are involved in the metabolism of ondansetron in humans.32 On the other hand, Gilbert et al.32 reported that a 17% increase in the clearance of cyclophosphamide was observed in the presence of continuous-infusion ondansetron during high-dose chemotherapy for breast cancer, suggesting the possibility of CYP induction.6

In conclusion, the present study suggests that azasetron, ondansetron, and ramosetron do not cause clinically significant interactions with other drugs that are metabolized by CYPs, such as CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, via the inhibition of metabolism.

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23) Dixon C. M., Colthup P. V., Serabji-Singh C. J., Kerr B. M., Boehliert


