Drug Interactions between Theophylline and H₂-Antagonists, Roxatidine Acetate Hydrochloride and Cimetidine: Pharmacokinetic Analysis in Rats in Vivo

Naomi Nagai, Miwa Furuhata, and Hiroyasu Ogata*

Department of Biopharmaceutics, Meiji College of Pharmacy, Yato 1-22-1, Tanashi, Tokyo 188, Japan.
Received April 19, 1995; accepted July 17, 1995

The effects of roxatidine acetate hydrochloride (Rox) on the pharmacokinetics of theophylline were compared to those of cimetidine in rats in vivo. Cimetidine or Rox was maintained at a steady state level using continuous infusion, then theophylline was injected into the rats as a bolus (7.5 mg/kg). Cimetidine showed competitive inhibition of theophylline elimination in vivo, with an inhibition constant (K_i) of 28.5 μM. Cimetidine significantly decreased the total body clearance and extended the plasma half-life of theophylline, but did not change its volume of distribution. In contrast, Rox did not significantly influence the pharmacokinetics of theophylline in rats. The in vivo animal model used in the present study for investigating the mechanism of the drug interaction showed good agreement with the results obtained in clinical and in vitro studies.

Key words drug interaction; H₂-antagonist; theophylline; roxatidine acetate hydrochloride; cimetidine; pharmacokinetics

Roxatidine acetate hydrochloride (Rox) is a histamine H₂-receptor antagonist used in the treatment of peptic ulcers and other acid-related disorders, with an inhibitory effect on stimulated gastric acid secretion approximately six-fold more potent than cimetidine. Cimetidine interacts with many drugs by inhibiting hepatic microsomal oxidation. This inhibition has been accounted for by its high affinity for cytochrome P450 arising from its imidazole and/or cyanogroups. Rox has no imidazole group in its chemical structure, and shows a different type of interaction with the hepatic microsome system from that of cimetidine.

Cimetidine is known to decrease theophylline clearance, however, Rox has not significantly influenced theophylline pharmacokinetics in humans. The drug interaction of H₂-antagonists has been kinetically investigated in in vitro studies. In the present study, the in vivo interactions between theophylline and the H₂-antagonists, cimetidine and Rox, were studied in rats.

MATERIALS AND METHODS

Materials Rox and cimetidine were generously provided by Teikoku Hormone Mfg. Co., Ltd. (Tokyo, Japan). Theophylline was purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All other chemicals and solvents were of analytical grade.

Pharmacokinetic Study Rox or cimetidine was dissolved in saline to obtain concentrations of 2 and 15 mg/ml (Rox), or 0.2 to 15 mg/ml (cimetidine) for intravenous infusion. After overnight fasting, male Wistar rats (209 ± 17.6 g) were anesthetized with ethyl carbamate (1 g/kg). A polyethylene catheter (PE-50) was inserted into the carotid artery for sampling blood and a second into the right jugular vein for infusion of H₂-antagonists. Steady state plasma H₂-antagonist levels were attained by continuous infusion of 20, 80 and 200 μg/min of cimetidine for 30 min after bolus injection (2, 8 and 15 mg/kg, respectively), and by continuous infusion of 80 μg/min Rox for 30 min after bolus injection (15 mg/kg). Saline, as a control, was continuously infused at 0.1 ml/min for 30 min. After 30 min infusion of H₂-antagonists or saline, theophylline was injected as a bolus (7.5 and 22.5 mg/kg) via the left femoral vein. Continuous infusion of H₂-antagonists or saline was continued until the final sampling.

Blood samples (0.3 ml) were collected in heparinized micro tubes before and 5, 15, 30, 60, 120 and 240 min after bolus injection of theophylline. The blood was immediately centrifuged (1000 × g) for 3 min at room temperature and the plasma samples obtained were stored at -20 °C until analysis.

Analysis Plasma concentration of theophylline was determined by reverse-phase high-performance liquid chromatography (HPLC) as reported previously, with slight modification. Fifty microliters of internal standard solution (20 μg/ml β-hydroxyethyl theophylline in methanol) and 0.2 ml of 0.067 M phosphate buffer (pH 8) were added to each 0.1 ml plasma sample. Theophylline was extracted by adding 5 ml chloroform and shaking for 10 min. After centrifugation (850 × g) for 5 min, 4 ml of the organic layer was transferred to another glass tube and evaporated to dryness at 40 °C under nitrogen. The residue was dissolved in 100 μl of mobile phase, and 20 μl of this solution was injected into the column. The HPLC system was the same as that used in the previous study.

The mobile phase consisted of 0.02 M acetic acid buffer (pH 5) and acetonitrile (93:7, v/v) and the flow rate was 1.5 ml/min. The UV wavelength for theophylline determination was 275 nm.

Plasma concentration of cimetidine was determined by reverse-phase HPLC with some modifications of the previous reports. Fifty microliters 2 M sodium hydroxide and 5 ml methylene chloride were added to 0.1 ml plasma. The mixture was then extracted by shaking for 10 min. After centrifugation (850 × g) for 5 min, 4 ml of the organic layer was transferred and evaporated to dryness at 40 °C under nitrogen. The residue was redissolved in 100 μl internal standard solution (2.5 μg/ml 1,3-dimethyluric acid in methanol) and 20 μl of this solution was injected into the column. The HPLC system

* To whom correspondence should be addressed.

© 1995 Pharmaceutical Society of Japan
used for cimetidine analysis was the same as that used for theophylline, except that the mobile phase comprised 0.01 M sodium acetate (pH 5.2) and methanol (3:1, v/v) and the flow rate was 1.2 ml/min. Cimetidine was monitored at 228 nm.

Plasma concentration of Rox was determined by gas chromatography-mass spectrometry. The extraction procedure, equipment and operating conditions were the same as those used in the previous study.12)

**Pharmacokinetic Analysis** The area under the plasma concentration-time curve from zero time to infinity (AUC), the total body clearance (Cl), the plasma half-life obtained from the terminal elimination phase (t1/2), and the volume of distribution at steady state (Vdss) were calculated using the least squares fit program, MULTI13) according to the following equations

\[
C_p = A e^{-\alpha t} + B e^{-\beta t} \quad (1)
\]

\[
AUC = A(\alpha + B/\beta) \quad (2)
\]

\[
t_{1/2} = 0.693/\beta \quad (3)
\]

\[
Cl = D_0 / AUC \quad (4)
\]

\[
V_{dss} = V_1 + V_2 \quad (5)
\]

where \( C_p \) is the theophylline concentration and \( A, \alpha, B \) and \( \beta \) are the biexponential equation constants. \( D_0 \) is the dose of theophylline and \( V_1 \) and \( V_2 \) are the volumes of distribution of the central and peripheral compartments, respectively. The inhibitory effect of cimetidine on the theophylline elimination in vivo was analyzed by the Dixon plot using \( C_p_0 \) (theophylline concentration at zero time) and \( Cl \) instead of in vitro metabolic rate (\( \eta \)).

**Statistical Analysis** The differences in pharmacokinetic parameters of theophylline in rats treated with four different steady-state plasma levels of cimetidine were analyzed by one-way ANOVA and subsequently by multiple range Tukey test.14) The differences in pharmacokinetic parameters of theophylline between treatments with Rox and saline were analyzed statistically using Student's t-test for unpaired data. Statistical significance was set at \( p < 0.05 \).

---

**RESULTS AND DISCUSSION**

The plasma concentration-time curves of theophylline after bolus injection (7.5 mg/kg) to rats under four different steady state plasma cimetidine levels 0 (control) and means of 10.4, 37.2 and 106.2 \( \mu \)g are shown in Fig. 1. Theophylline elimination was delayed according to the increase in the steady-state plasma cimetidine level.

The effect of steady-state plasma cimetidine levels on the pharmacokinetic parameters of theophylline are shown in Table 1. When rats were treated with the highest level of cimetidine (106.2 \( \mu \)g), \( Cl \) and \( \beta \) were significantly reduced to 28.3\% and 29.1\%, and \( t_{1/2} \) and \( AUC \) increased 3.6- and 3.5-fold, respectively. However, \( \alpha \), \( C_p_0 \), \( V_1 \), \( V_2 \) and \( V_{dss} \) were not significantly affected by cimetidine treatment.

In Fig. 2, the reciprocal of the theophylline elimination rate, represented using both \( Cl \) and the estimated plasma concentration of theophylline at time zero (\( C_p_0 \)) are plotted against the steady state plasma cimetidine levels, which corresponds to a Dixon plot. The plot shows competitive inhibition of cimetidine for theophylline elimination, and the obtained in vivo inhibition constant (\( K_i \)) is 28.5 \( \mu \)g for rats.

---

**Table 1. Effect of Steady State Plasma Cimetidine Levels on Pharmacokinetic Parameters of Theophylline in Rats after Bolus Injection of Theophylline (7.5 mg/kg)**

<table>
<thead>
<tr>
<th>Mean cimetidine level (( \mu )g)</th>
<th>A 0</th>
<th>B 10.4</th>
<th>C 37.2</th>
<th>D 106.2</th>
<th>ANOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha ) (h(^{-1}))</td>
<td>4.51±4.21</td>
<td>6.05±1.07</td>
<td>9.92±3.72</td>
<td>5.82±2.71</td>
<td>N.S.</td>
</tr>
<tr>
<td>( \beta ) (h(^{-1}))</td>
<td>0.327±0.027</td>
<td>0.232±0.066</td>
<td>0.136±0.013</td>
<td>0.095±0.025</td>
<td>A&gt;B&gt;C&gt;D</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>2.12±0.17</td>
<td>3.18±1.03</td>
<td>5.12±0.51</td>
<td>7.72±2.42</td>
<td>D&gt;C&gt;B&gt;A</td>
</tr>
<tr>
<td>( C_p_0 ) (mg/ml)</td>
<td>12.43±2.18</td>
<td>14.35±1.46</td>
<td>10.45±1.20</td>
<td>11.70±1.29</td>
<td>N.S.</td>
</tr>
<tr>
<td>( AUC ) (mg/ml\cdot h)</td>
<td>26.1±2.9</td>
<td>38.5±13.8</td>
<td>56.6±6.7</td>
<td>92.4±12.0</td>
<td>D&gt;C&gt;B&gt;A</td>
</tr>
<tr>
<td>( Cl ) (l/h/kg)</td>
<td>0.290±0.034</td>
<td>0.188±0.045</td>
<td>0.134±0.016</td>
<td>0.082±0.011</td>
<td>A&gt;B&gt;C&gt;D</td>
</tr>
<tr>
<td>( V_1 ) (l/kg)</td>
<td>0.62±0.10</td>
<td>0.53±0.05</td>
<td>0.72±0.08</td>
<td>0.65±0.08</td>
<td>N.S.</td>
</tr>
<tr>
<td>( V_2 ) (l/kg)</td>
<td>0.17±0.08</td>
<td>0.28±0.08</td>
<td>0.26±0.04</td>
<td>0.24±0.09</td>
<td>N.S.</td>
</tr>
<tr>
<td>( V_{dss} ) (l/kg)</td>
<td>0.79±0.02</td>
<td>0.80±0.12</td>
<td>0.98±0.12</td>
<td>0.89±0.17</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters were calculated using the equations described in the text. Values are expressed as mean (n=3) ±S.D. a) Treatments underlined by a common line did not differ significantly by Tukey test (\( p < 0.05 \)).
for mostly by 8-hydroxylation and N-methylation. In our previous clinical study, we demonstrated the inhibitory effect of cimetidine, but no effect of Rox on theophylline clearance in healthy subjects. In the present in vivo animal study, cimetidine significantly reduced the theophylline clearance, and the pattern of Dixon plot characterized the in vivo inhibitory effect of cimetidine on theophylline elimination as the competitive type. This in vivo interaction between the two drugs is consistent with the results obtained in human and in vitro studies.

The Ki value calculated using the Dixon plot (Fig. 2) was 28.5 μM in rats in vivo. At least two P450 isozymes contributed to theophylline metabolism, one corresponding to CYP1A and the other being associated with a phenobarbital-inducible P450. Cimetidine inhibited N-demethylation and 8-hydroxylation of theophylline in human microsomes with Ki 0.5—1.0 nm range. The in vivo plasma concentrations of cimetidine are in the range of 3–10 μM in humans; this is lower than the in vitro Ki values. The similar tendency is observed in rats, comparing the present results with previous in vitro microsomal studies. The reason for this discrepancy has been previously suggested that the cimetidine concentration required for in vitro inhibition is probably attained locally in the hepatic endoplastic reticulum.

Rox has a chemical structure quite different from that of cimetidine, which suggests that it will have a less inhibitory potency on the oxidative drug-metabolizing enzymes. Rox gave an absorption maximum at 418 nm and a minimum at 384 nm, showing a reverse type-I spectrum, in contrast to the type-II spectrum of cimetidine. The spectral dissociation kinetics indicated that the affinity of Rox for cytochrome P450 was about one fifth that of cimetidine, and Rox also showed weaker inhibitory effects on in vitro testosterone hydroxylation, aminopyrine N-demethylation and aniline hydroxylation.

The interaction between Rox and theophylline was characterized under similar in vivo conditions where Rox was maintained at a steady-state level (mean 20.0 μM). Mean plasma concentration against time and pharmacokinetic parameters of theophylline after bolus injection (7.5 mg/kg) under steady state plasma levels of Rox (means 0 and 20.0 μM) are shown in Fig. 3 and Table 2. Rox had no effect on theophylline plasma concentration or pharmacokinetic parameters. The peak plasma level of Rox after oral dose has been in the range of 0.5—1 μM in a clinical setting, which is lower than cimetidine. The present results were in good agreement with those obtained in clinical and in vitro studies.

REFERENCES