Lack of Pharmacokinetic Interaction between Pilsicainide and Rifampicin in Healthy Volunteers

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Pilsicainide, a class Ic antiarrhythmic agent, is a cationic compound. It has been hypothesized that a P-glycoprotein (P-gp)-induced transport mechanism may mediate the intestinal absorption and the renal excretion of pilsicainide. We evaluated whether rifampicin, a known inducer of P-gp, affects the pharmacokinetics of pilsicainide after oral dosing in healthy subjects. A pharmacokinetic study was conducted on 8 healthy male subjects (aged 30 ± 8 years; body weight 65.7 ± 6.5 kg) and demonstrated that rifampicin (450 mg given orally once daily for 4 days) did not significantly affect the maximum plasma concentration (pilsicainide alone: 0.39 ± 0.15 versus pilsicainide + rifampicin: 0.36 ± 0.06 μg/mL), the time to maximum plasma concentration (1.38 ± 0.83 versus 1.06 ± 0.18 h), the area under the plasma concentration-time curve (2.81 ± 0.91 versus 2.58 ± 0.62 μg·h/mL), the renal clearance (198.46 ± 85.93 versus 194.34 ± 69.91 mL/min) or the net renal clearance by tubular secretion (128.75 ± 73.56 versus 119.93 ± 79.84 mL/min) of pilsicainide after a single oral dose (50 mg). In conclusion, our results indicated that rifampicin did not affect the pharmacokinetics of pilsicainide after oral dosing in humans.

Key words: pilsicainide, rifampicin, pharmacokinetics, interaction

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In our previous study, we found that coadministration of verapamil with pilsicainide shortened the time to peak concentration and tended to increase the peak concentration of pilsicainide, although the differences were small\textsuperscript{11}. Furthermore, an interesting case report of a patient who received both rifampicin (450 mg daily) and pilsicainide (50 mg three times daily) showed that the blood concentration of pilsicainide was undetectable (<0.05 mg/mL) even 90 minutes after oral administration in the morning\textsuperscript{12}. Rifampicin is a known inducer of P-gp mediated by activation of the nuclear pregnane X receptor\textsuperscript{13,14}. P-glycoprotein plays a major role in the rate and the extent of absorption of several drugs from the gastrointestinal tract and in the renal secretion of many drugs\textsuperscript{15}. Therefore, rifampicin may inhibit the oral absorption of pilsicainide by inducing the intestinal efflux transport of this drug via P-gp. However, few studies have evaluated this issue.

The aim of this study was to evaluate whether rifampicin affects the pharmacokinetics of pilsicainide after oral dosing in healthy subjects.

**Methods**

A total of 8 healthy male volunteers (30±8 years of age; body weight 65.7±6.5 kg) were enrolled in this study. None of the subjects had a history of cardiovascular, renal, or hepatic disease. The physical examinations, electrocardiographic readings, and laboratory screening tests for each subject were normal before the subjects were included in the study. The study protocol was approved by the institutional review boards at Tokyo Women’s Medical University and Clinical Research Hospital Tokyo. This study was conducted according to the Declaration of Helsinki, and all of the subjects provided written informed consent.

This study was designed as an open-label, two-phase, fixed-order study. On day 1, all the subjects were admitted and not allowed any food or liquid for more than 9 hours before the study and for 4 hours after drug administration. Each subject received a 50-mg dose of pilsicainide hydrochloride (Sunrythm® 50-mg capsule, Daiichi-Sankyo, Tokyo, Japan) orally at 9 am on day 2. Blood (10 mL) was drawn into heparinized tubes from a forearm vein before administration of the drug and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 12, and 24 hours after drug administration. The blood samples were centrifuged (1,450×g, 10 minutes, 4°C) immediately, and the plasma was stored in plastic vials at −20°C until analysis. Urine samples were collected and stored in plastic bottles for the following time intervals: 0-1, 1-2, 2-3, 3-4, 4-8, 8-16, and 16-32 hours after drug administration.

After a washout period of 7 days, the subjects were readmitted on day 9 and received 450 mg of rifampicin (Rifadin® 150-mg capsule, Daiichi-Sankyo, Tokyo, Japan) orally once daily for 4 days (day 10 to day 13)\textsuperscript{16,17}. On day 13, the subjects were coadministered 50 mg of pilsicainide and 450 mg of rifampicin at 9 am. Blood and urine samples were collected using the same methodology as on day 2.

**Determination of pilsicainide in plasma and urine**

The concentrations of pilsicainide in plasma and urine were measured using a liquid chromatography-mass spectrometry (LC-MS) system. For sample preparation, 0.1 mL of plasma sample, an ultrafiltrate of plasma sample (i.e., protein-free plasma) or a three- to ten-fold dilution of urine sample was mixed with 0.1 mL of 0.5 M sodium carbonate and 1 mL of diethyl ether. After the mixture was vortex-mixed for 30 seconds and centrifuged for 5 minutes at 9,000×g, the organic layer was separated. The diethyl ether layer was evaporated to dryness at 40°C under a stream of nitrogen gas.

The residue was reconstituted with 0.25 mL of the mobile phase, and a 2-μL sample was injected into the LC-MS system. The LC-MS system consisted of a 3200 QTRAP LC/MS/MS system (AB SCIEX, Tokyo, Japan) with a LC-20AD Prominence Liquid Chromatograph, a DGU-20A3 Prominence Degasser, a CTO-20A Prominence Column Oven, a SIL-20AC HT Prominence Autosampler and a CBM-20A Prominence Communications Bus Module (Shimadzu, Kyoto, Japan). The analytes were separated on an L-column (75 mm×2.1 mm i.d., particle size 2 μm, CERI, Saitama, Japan), which was preceded by a pre-column filter. The temperature of the column was maintained at 25°C. The samples were isocratically eluted using a mobile phase composed of 5 mM ammonium acetate and acetonitrile (4:1, v/v) at a flow rate of 0.2 mL/min.

The MS conditions were as follows: curtain gas 40, collision gas 4, ion spray voltage 5,500 V, temperature 700°C, ion source gas 1 70 psi, ion source gas 2 70 psi, declustering potential 70 V, entrance potential 10 V.
collision energy 50 V, and collision cell exit potential 3 V. A positive ion mode was used, and selected-ion monitoring was accomplished at m/z 273 for pilsicainide and m/z 325 for quinidine as an internal standard.

Standard curves were constructed for pilsicainide concentrations ranging from 0.025 to 1 µg/mL in plasma, protein-free plasma, and urine. Using authentic samples, the total recovery of pilsicainide was more than 80% from plasma, protein-free plasma and urine. The within-day and day-to-day variabilities measured using authentic samples were below 9.7% and 7.8% in plasma (n=5), below 8.8% and 7.5% in protein-free plasma (n=5), and below 4.6% and 10.0% in urine (n=5). The detection limit for pilsicainide was estimated to be 0.001 µg/mL. Pilsicainide plasma protein binding experiment was conducted using a microfiltration and ultrafiltration system Centricut V-10 (Kurabo Industries Ltd., Osaka, Japan) by centrifugation for 5 minutes at 1,450×g and 25°C.

**Pharmacokinetic analysis**

The peak concentration (C_{max}) and the time to peak concentration (t_{max}) were determined using the observed values. The area under the plasma concentration-time curve (AUC) from 0 to the last sampling time (AUC_{0−t}) was determined using the trapezoidal rule. The AUC from 0 to infinity (AUC_{0−∞}) was calculated using the following equation:

\[ AUC_{0−∞} = AUC_{0−t} + C_{pt}/\beta \]

where \( C_{pt} \) and \( \beta \) represent the observed plasma concentration at the last sampling time (t) and the elimination rate constant at the terminal phase obtained by a least squares linear regression analysis, respectively. The apparent total clearance after oral administration (CL/F) was determined using the following equation:

\[ CL/F = \text{Dose}/AUC_{0−∞} \]

The apparent fraction of dose excreted unchanged in the urine (F×fe) was calculated as the amount of pilsicainide recovered in the urine divided by the amount of pilsicainide administered (50 mg). The unbound fraction in plasma (fu) was expressed as the quotient of the ultrafiltered concentration divided by the prefiltered plasma concentration. The fractional renal clearance (CLR) (0–1, 1–2, 2–3, 3–4, 4–8 hours) was expressed as the quotient of the amount recovered in the urine divided by the AUC of pilsicainide for the corresponding periods. The CLF over the period from 0 to 32 hours was calculated as the quotient of the cumulative amount of pilsicainide in urine divided by plasma AUC_{0−∞}. The net CLF of tubular secretion (CL_{TS}) was calculated (supposing that the reabsorption of pilsicainide can be ignored) using the following formula:

\[ CL_{TS} = CL_{F} - (fu \times CL_{CR}) \]

where CL_{CR} obtained using 24-hour urine sample and serum creatinine was calculated as the urinary clearance rate per min divided by serum creatinine concentration.

**Statistical analysis**

The data are expressed as mean ± standard deviation (SD). The statistical significance of the differences in pharmacokinetic parameters between two time points was estimated using a paired t-test. A p value < 0.05 was considered statistically significant.

**Results**

Figure 1 shows the plasma concentration-time curves for pilsicainide when administered alone and when coadministered with rifampicin. There were no significant differences in mean plasma concentrations of pilsicainide between the 2 administrations. Table summarizes the mean pharmacokinetic parameters of
pilsicainide given with and without rifampicin. Coadministration of rifampicin did not significantly alter any of the pharmacokinetic parameters of pilsicainide and did not affect the CLs or the net CLts of pilsicainide. Figure 2 shows the hourly CLs (0–4 hours), the 4-hour CLs (4-8 hours), and the total CLs (0–32 hours) of pilsicainide administered alone or coadministered with rifampicin. Coadministration of pilsicainide with rifampicin did not significantly alter the CLs values of pilsicainide at each of the time points. No subjects experienced any adverse events throughout the course of the study.

### Discussion

Our human study revealed that rifampicin did not change the tmax, the Cmax, or the AUC of pilsicainide, and did not significantly affect the renal tubular excretion of pilsicainide in healthy subjects. These results suggest that rifampicin does not decrease the oral absorption of pilsicainide or the elimination of pilsicainide.

These results do not explain a previous case report in which blood pilsicainide concentration was undetectable in a patient receiving rifampicin and pilsicainide\(^{12}\). However, that case report had several limitations, such as only one determination of blood concentration, no reexamination of blood concentration after coadministration with rifampicin at another time point, and no confirmation of drug administration, especially pilsicainide.

In humans, approximately 90% of orally administered pilsicainide is recovered in the urine, of which 75%–86% is in the unchanged form\(^{8}\). The remaining pilsicainide is metabolized to its main metabolite, 2-hydroxymethylate, via cytochrome P450 (CYP)2D6\(^{18}\), only 5% of which is recovered from the urine\(^{8}\). Rifampicin does not produce clinically significant induction of CYP2D6\(^{19}\). We could not measure the total amount of absorbed pilsicainide because the metabolite was not measured in this study. However, rifampicin did not affect the pharmacokinetic profile of the unchanged drug. Therefore, our results suggest

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pilsicainide alone</th>
<th>Pilsicainide + Rifampicin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (μg/mL)</td>
<td>0.39 ± 0.15</td>
<td>0.36 ± 0.06</td>
<td>0.46</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>1.38 ± 0.83</td>
<td>1.06 ± 0.18</td>
<td>0.31</td>
</tr>
<tr>
<td>AUC0-∞ (μg*h/mL)</td>
<td>2.81 ± 0.91</td>
<td>2.58 ± 0.62</td>
<td>0.19</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>16.84 ± 5.71</td>
<td>17.42 ± 4.07</td>
<td>0.59</td>
</tr>
<tr>
<td>Vd/F (L)</td>
<td>118.31 ± 27.94</td>
<td>117.71 ± 31.60</td>
<td>0.92</td>
</tr>
<tr>
<td>Elimination t1/2 (h)</td>
<td>5.20 ± 1.92</td>
<td>4.85 ± 0.82</td>
<td>0.41</td>
</tr>
<tr>
<td>f(u) (%)</td>
<td>57.02 ± 10.53</td>
<td>57.49 ± 10.68</td>
<td>0.82</td>
</tr>
<tr>
<td>Ae (%)</td>
<td>68.72 ± 15.85</td>
<td>65.44 ± 13.73</td>
<td>0.49</td>
</tr>
<tr>
<td>CLs (mL/min)</td>
<td>198.46 ± 85.93</td>
<td>194.34 ± 69.91</td>
<td>0.79</td>
</tr>
<tr>
<td>CLcr (mL/min)</td>
<td>130.80 ± 25.43</td>
<td>128.30 ± 13.99</td>
<td>0.80</td>
</tr>
<tr>
<td>CLts (mL/min)</td>
<td>128.75 ± 73.56</td>
<td>119.93 ± 79.84</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Values are means ± SD.

Cmax, maximum plasma concentration; tmax, time to reach Cmax; AUC0-∞, area under the plasma concentration-time curve from 0 to infinity; CL/F, apparent total clearance; Vd/F, apparent volume of distribution; t1/2, half-life; f(u), unbound fraction in plasma; Ae, absorbed dose excreted unchanged in urine; CLs, renal clearance; CLcr, creatinine clearance; CLts, net renal clearance by tubular secretion.
that rifampicin has no clinical effect on the intestinal absorption of pilsicainide.

In our study, rifampicin did not affect the elimination of pilsicainide. We previously reported that renal elimination of pilsicainide was not inhibited by verapamil, a potent P-gp inhibitor, in human and experimental studies. Tsuruoka et al. suggested that P-gp-mediated transport and cation transport may contribute to renal excretion of pilsicainide. However, P-gp-mediated transport does not contribute to the elimination of pilsicainide in humans, at least not clinically, because renal elimination of pilsicainide was not increased by rifampicin, which is a potent inducer of P-gp.

In conclusion, this human study demonstrated that the potent P-gp inducer rifampicin did not affect the pharmacokinetics of pilsicainide after oral dosing. Our results suggest that P-gp-mediated transport does not contribute to intestinal absorption or renal tubular secretion of pilsicainide in humans.

Conflicts of Interest
Dr. Shiga received scholarship funds from Daiichi-Sankyo. The remaining authors declare no conflicts of interest.

References