Note

Effect of Benidipine on Simvastatin Metabolism in Human Liver Microsomes

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Summary: Benidipine, which is a calcium channel blocker that has clinical advantages in the treatment of hypertension, is metabolized by CYP3A4 in humans. The effect of benidipine on the metabolism of simvastatin by human liver microsomes was investigated in order to predict the potential of in vivo drug-drug interactions between benidipine and other substrates of CYP3A4. The results were compared with data generated with azelnidipine, which is also metabolized by CYP3A4.

Both benidipine and azelnidipine inhibited simvastatin metabolism in vitro in a concentration-dependent manner. Assuming competitive inhibition, the Ki values based on the unbound concentrations, were calculated to be 0.846 and 0.0181 μM for benidipine and azelnidipine, respectively. If simvastatin (10 mg) and benidipine (8 mg, the clinically recommended highest dose) were to be administered concomitantly, the ratio of the areas under the concentration-time curves of simvastatin with and without benidipine (AUC(+I)/AUC) was predicted to be 1.01. On the other hand, if simvastatin (10 mg) and azelnidipine (8 mg) were co-administered, the AUC(+I)/AUC for simvastatin was predicted to be 1.72, which is close to the observed value (1.9) in healthy volunteers. These data suggest that benidipine is unlikely to cause a drug interaction by inhibiting CYP3A4 activity in the liver.

Key words: drug interactions; CYP3A4; in vitro-in vivo prediction; inhibition; liver microsomes

Introduction

Benidipine (Coniel®, Kyowa Hakko Kogyo, Tokyo, Japan, Fig. 1), is a 1,4-dihydropyridine calcium channel blocker that is used successfully to treat hypertension and angina pectoris. Compared with earlier dihydropyridine-based calcium antagonists (e.g. nifedipine, nicardipine and nitrendipine), benidipine has an improved pharmacodynamic profile; its effects are slower in onset and more sustained.1–3

Azelnidipine (Calblock®, Sankyo, Tokyo, Japan, Fig. 1) is also a calcium channel blocker; it has a long elimination half-life (t1⁄2) and has been classified as a third generation calcium antagonist.6 The main CYP isozyme involved in azelnidipine metabolism in human liver is CYP3A4.3–5

Simvastatin (Fig. 1), an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, is widely used in the treatment of hypercholesterolemia.6–9 Simvastatin is rapidly absorbed after oral administration to humans and is extensively metabolized by the hepatic first-pass system. Prueksaritanont et al. reported that metabolism of simvastatin by human liver microsomes was inhibited strongly by troleandomycin indicating that CYP3A was the main enzyme involved in the transformation of the drug.9 Metabolism of simvastatin was also weakly inhibited by diethylthiocarbamate, which indicated a minor involvement of CYP2E1 and 2B6.8

Drug-drug interactions caused by the inhibition of drug-metabolizing enzymes such as cytochrome P450 (CYP) isozymes are common9,10 and can lead to serious toxicities.11 Co-administration of simvastatin with itraconazole, an antifungal agent, can result in rhabdomyolysis. This adverse event has been attributed to an increase in simvastatin blood levels caused by potent inhibition of CYP3A4-mediated metabolism of simvastatin by itraconazole.12 Ishigami et al. used in vitro metabolic data to quantitatively predict in vivo drug-drug interactions between simvastatin and itraconazo-
The predicted in vivo interaction agreed well with the effect observed in clinical studies.13) In the clinic, it is common practice for simvastatin to be co-administered with 1,4-dihydropyridine calcium antagonists and, therefore, it is necessary to be aware of possible drug-drug interactions. When healthy subjects received 10 mg of simvastatin together with 8 mg of azelnidipine, exposure to simvastatin was increased by 1.9 times compared with data when simvastatin alone was administered.4) These results suggested that azelnidipine competitively inhibits CYP3A4-mediated metabolism of simvastatin. Benidipine is also metabolized by CYP3A4 in humans (Asanome, K., Kajita, J., Inano, K., Kobayashi, H., unpublished data), however, there are no published reports regarding the effect of benidipine on simvastatin metabolism. Therefore, it is important to predict a potential quantitative change in the pharmacokinetics of simvastatin.

In the present study, we investigated the effect of benidipine on simvastatin metabolism by human liver microsomes in vitro and compared the results to the effect of azelnidipine. In addition, we predicted the in vivo drug-drug interactions that would occur if simvastatin and benidipine, or azelnidipine, were co-administered, based on CYP3A4 inhibition in the liver.

Materials and Methods

Materials: Benidipine, azelnidipine and lovastatin were synthesized at Kyowa Hakko Kogyo. Simvastatin was from Wako Pure Chemical Industries (Osaka, Japan). Beta-nicotinamide-adenine dinucleotide phosphate, reduced form (β-NADPH), was from Oriental Yeast (Tokyo, Japan). A mixed pool of human liver microsomes (20 mg protein/mL) prepared from 30 subjects was from XenoTech (Lenexa, KS, USA). Other chemicals were of the highest grade commercially available.

Inhibition Studies in Human Liver Microsomes: Since many metabolites are produced from simvastatin,6,8,14) we monitored simvastatin depletion with time to obtain in vitro Ki values in human liver microsomes rather than using the more traditional metabolite formation approach. The incubation times and protein concentration used were within the linear range for the metabolism of simvastatin.

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Binding to Microsomes: The binding of benidipine and azelnidipine to human liver microsomes was determined by equilibrium dialysis. The incubation mixture consisted of 1–35 μM benidipine or 2–35 μM azelnidipine, 0.1 mg protein/mL human liver microsomes, 100
mM phosphate buffer (pH 7.4) and 6 mM MgCl₂. The concentration of solvent in the incubation mixture was consistent with that used in the inhibition studies. The incubation buffer consisted of 100 mM phosphate buffer (pH 7.4) and 6 mM MgCl₂. The incubation mixture and the dialysis buffer consisted of 100 mM phosphate buffer concentration of solvent in the incubation mixture was monitored were ionization in the positive mode. The ion transitions Mass spectral analysis was performed using electrospray by high-performance liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS).

HPLC Analysis for Simvastatin: HPLC was carried out using a YMC-Pack Pro-C18 column (150 × 4.6 mm, 5 μm, YMC, Kyoto, Japan) and a mobile phase consisting of 34% (v/v) 10 mM ammonium acetate buffer (pH 4.5) and 66% (v/v) acetonitrile at a flow rate of 1 mL/min. The eluate was detected at a wavelength of 240 nm.

LC/MS/MS Analysis for Benidipine and Azelnidipine: A Symmetry C18 column (150 × 2.1 mm, 5 μm, Waters, Milford, MA, USA) was used with an Opti-Guard C18 guard cartridge (1 mm, Tomsci, Tokyo, Japan). The mobile phase consisting of 34% (v/v) 10 mM ammonium acetate buffer (pH 4.5) and 66% (v/v) acetonitrile was pumped at a flow rate of 0.2 mL/min. Mass spectral analysis was performed using electrospray ionization in the positive mode. The ion transitions monitored were m/z 506.3 to m/z 174.3 for benidipine, m/z 583.1 to m/z 167.1 for azelnidipine and m/z 405 to m/z 199 for lovastatin.

Data Analysis: The unbound fraction of each inhibitor in microsomes (fₙ(um)) was calculated using Eq. (1):

\[ f_{u(m)} = \frac{C_{\text{buffer}}}{C_{\text{incubation mixture}}} \]  

where \( C_{\text{buffer}} \) is the concentration of drugs in the buffer compartment and \( C_{\text{incubation mixture}} \) is the concentration of drugs in the incubation mixture compartment. Unbound concentrations of the inhibitors (Iₙ(um)) in the incubation mixture were calculated using Eq. (2).

\[ I_{u(m)} = \text{nominal} \cdot f_{u(m)} \]  

The kinetic data sets were evaluated for the type of inhibition by Lineweaver-Burk graphical analysis and competitive inhibition was chosen. The kinetic parameters were calculated from a model based on competitive inhibition using WinNonlin software (Pharsight, Mountain View, CA, USA). The velocity (v) was expressed as follows:

\[ v_{(+I)} = \frac{V_{\text{max}} \cdot S}{K_m (1 + \frac{I_{u(m)}}{K_i})} + S \]  

where S is the concentration of substrate. The subscript (+I) indicates the value calculated in drug-drug interaction experiments, i.e. plus inhibitor. The initial values of Michaelis-Menten constant (Kₘ) and maximum metabolic reaction velocity (Vₘₐₓ) were calculated from Lineweaver-Burk plots and the initial Kᵢ value was calculated using Dixon plots.

Quantitative Prediction of In Vivo Drug-Drug Interactions: The areas under the plasma concentration-time curves of simvastatin in the presence and absence of test compounds (AUC(+I), AUC) after single oral administration of simvastatin were estimated using the “well-stirred” model using Eq. (4). For these calculations, it was assumed that the liver is the only organ involved in simvastatin clearance and that protein binding of simvastatin is not affected by the addition of benidipine or azelnidipine.

\[ \frac{\text{AUC}(+I)}{\text{AUC}} = \frac{\text{CL}_{\text{int}} \cdot D(+I)}{\text{CL}_{\text{int}} (+I)} = \frac{\text{CL}_{\text{int}}}{\text{CL}_{\text{int}} (+I)} \]  

where \( \text{CL}_{\text{int}} \) is the liver intrinsic clearance and D is the dose.

When the substrate concentration is much lower than \( K_m \) (\( K_m \gg S \)), \( \text{CL}_{\text{int}} \) and \( CL_{\text{int} (+I)}/CL_{\text{int}} \) values can be expressed as in Eqs. (5) and (6), except if inhibition is uncompetitive.

\[ \text{CL}_{\text{int}} = \frac{V_{\text{max}}}{K_m} \]  

\[ \frac{\text{CL}_{\text{int} (+I)}}{\text{CL}_{\text{int}}} = \frac{1}{1 + \frac{I_{u(m)}}{K_i}} \]  

In clinical situations, simvastatin is used at a maximum dose of 40 mg and with this dose the unbound concentration of simvastatin in plasma (0.5 nM) is much less than \( K_m \).

The plasma unbound concentration at the entrance to the liver (Cₐ), where the blood flow from the hepatic artery and the portal vein meet, was considered to be the maximum value of Iₙ (Cₐ = Iₙ). Iₙ was calculated using Eqs. (7) and (8):

\[ C_{\text{in}} \leq I_{\text{max}} + \frac{k_s \cdot D \cdot F_a}{Q_h \cdot R_b} \]  

\[ I_{\text{u}} = C_{\text{in}} \cdot f_{u(p)} \]  

where \( I_{\text{max}} \) is the maximum plasma concentration in the systemic circulation, \( k_s \) is the absorption constant, \( F_a \) is
the fraction absorbed from the gastrointestinal tract into the portal vein, \( Q_h \) is the blood flow in the hepatic vein (1610 mL/min),\(^{19}\) \( R_B \) is the blood to plasma concentration ratio and \( f_{\text{u}(p)} \) is the unbound fraction of the drug in plasma. The \( I_u \) value of benidipine was calculated using the \( I_{\text{max}} \) (3.89 ng/mL)\(^{20}\) obtained with a single oral dose of 8 mg was co-administered with simvastatin in an \textit{in vivo} drug-drug interaction study. The \( I_{\text{max}} \) (13.1 ng/mL) determined after a single oral dose of 15 mg azelnidipine in a Phase I study,\(^4\) and the \( I_{\text{max}} \) (48.3 ng/mL) obtained with repeated administration of 16 mg once daily for 6 weeks to patients\(^{21}\) were used in the calculations. The \( k_a \) (0.51 h\(^{-1} = 0.0086 \text{ min}^{-1} \) ) of azelnidipine was calculated from \( t_{\text{max}} \) (2.3 h) and the elimination rate constant \( (k_{\text{el}}; \ln 2/t_{1/2} = 0.693/1.9) \) obtained after a single dose of 15 mg\(^{4}\) using Eq. (9). The \( R_B \) of azelnidipine, 0.62, was estimated using the blood cell distribution (RBC; 27.2\%).\(^4\) The \( f_{\text{u}(p)} \) of azelnidipine, 0.095, was calculated from data obtained in an \textit{in vitro} study.\(^4\) For both benidipine and azelnidipine was assumed to be 1, in order to make the \( I_u \) values maximum and avoid underestimation of a drug-drug interaction.

**Results**

**Inhibition Studies in Human Liver Microsomes:** The \( f_{\text{u}(p)} \) values of benidipine and azelnidipine were 0.111–0.140 and 0.00173–0.00360, respectively. Plots of the metabolic activity of simvastatin (2, 4, 10, 20 and 50 \( \mu \text{M} \) ) in microsomes in the presence of 0, 1, 10, 20 and 35 \( \mu \text{M} \) benidipine (unbound concentration 0, 0.139, 1.40, 2.50 and 3.89 \( \mu \text{M} \) ) or 0, 2, 10, 20 and 35 \( \mu \text{M} \) azelnidipine (unbound concentration 0, 0.00720, 0.0211, 0.0382 and 0.0606 \( \mu \text{M} \) ) are shown in Fig. 2. The \( K_i \) value of benidipine was 0.846 \( \mu \text{M} \), which was 47 times higher than that of azelnidipine (0.0181 \( \mu \text{M} \) ). The kinetic parameters \( (K_m, V_{\text{max}}) \) for simvastatin in both inhibition studies were similar: the \( K_m \) values for simvastatin in the inhibition studies with benidipine and azelnidipine were 6.78 and 4.62 \( \mu \text{M} \), respectively, and the \( V_{\text{max}} \) values were 12.1 and 10.3 nmol/min/mg protein, respectively.

**Quantitative Prediction of the \textit{in Vivo} Drug-Drug Interactions:** The parameters obtained from both \textit{in vitro} drug inhibition experiments and \textit{in vivo} pharmacokinetic analysis enabled us to predict that the AUC\(_{(\text{h})}/\text{AUC}\) of simvastatin (40 mg) co-administered with 8 mg of benidipine, the clinically recommended highest dose in humans, to simvastatin administered alone would be 1.01 (Table 1). In comparison, the
predicted AUC(\(\text{simvastatin}\))/AUC of simvastatin co-administered with a single dose of 8 mg azelnidipine and with repeat administration of 16 mg azelnidipine, the clinically recommended dose,\(^{21}\) was 1.72 and 2.68, respectively (Table 1).

### Discussion

It was reported that CYP3A4-mediated simvastatin metabolism was competitively inhibited by itraconazole;\(^{13}\) the predicted AUC(\(\text{simvastatin}\))/AUC was calculated to be 5.8 when the potential for microsomal binding of itraconazole was not considered.\(^{13}\) However, a clinical itraconazole-simvastatin interaction study\(^{9}\) revealed a much larger effect (AUC increased 19 times) than anticipated based on in vitro data.\(^{13}\) The discrepancy between the in vitro and in vivo data was attributed to the generation of invalid kinetic parameters in vitro due to the binding of itraconazole to microsomes.\(^{13}\) Therefore, we considered that it was necessary to determine \(f_{\text{u(sm)}}\) of the drugs in order to calculate valid \(K_i\) values.

When making predictions of in vivo drug-drug interactions based on in vitro results, the value of \(I_u\), which is the unbound concentration of the putative inhibitor in the liver, is required. In order to avoid an underestimate of \(I_u\), the unbound drug concentration in plasma at the entrance to the liver, where the blood flow from the hepatic artery and the portal vein meet, is used as the maximum value of \(I_u\).\(^{13,19}\) The concentrations of benidipine and azelnidipine derived from absorption after 8 mg oral doses were calculated \(I_{\text{max}} = \frac{k_s \cdot D \cdot F_s}{Q_h \cdot R_h}\) to be 0.378 and 0.118 \(\mu\text{M}\), respectively; these values were 53 and 6 times higher than \(I_{\text{max}} = 0.00501\) and 0.0130 \(\mu\text{M}\), respectively. If the maximum unbound concentration in the systemic blood circulation had been used as the value of \(I_u\), this would have led to an underestimate of the inhibition. In our study, using the calculated maximum concentrations of the drugs at the entrance to the liver, the predicted simvastatin AUC ratios (AUC(\(\text{simvastatin}\))/AUC) for benidipine or azelnidipine were 1.01 and 1.72, respectively.

### Table 1. Quantitative prediction of in vivo drug-drug interactions in humans based on in vitro data obtained from inhibition studies of simvastatin metabolism by benidipine or azelnidipine

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>(I_{\text{max}}) (ng/mL)</th>
<th>(I_{\text{max}}) ((\mu\text{M}))</th>
<th>(C_{\text{in}}) ((\mu\text{M}))</th>
<th>(f_{\text{u(sm)}})</th>
<th>(I_u) ((\mu\text{M}))</th>
<th>(K_i) ((\mu\text{M}))</th>
<th>(CL_{\text{int}(+I)} / CL_{\text{int}})</th>
<th>Prediction in vivo</th>
<th>Observation in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benidipine</td>
<td>8(^a)</td>
<td>3.89</td>
<td>0.00718</td>
<td>0.385</td>
<td>0.013</td>
<td>0.00501</td>
<td>0.846</td>
<td>0.994</td>
<td>1.01</td>
</tr>
<tr>
<td>Azelnidipine</td>
<td>8(^a)</td>
<td>11.2</td>
<td>0.0192</td>
<td>0.137</td>
<td>0.095</td>
<td>0.0130</td>
<td>0.0181</td>
<td>0.582</td>
<td>1.72</td>
</tr>
<tr>
<td>Azelnidipine</td>
<td>15(^b)</td>
<td>13.1</td>
<td>0.0225</td>
<td>0.244</td>
<td>0.095</td>
<td>0.0232</td>
<td>0.0181</td>
<td>0.439</td>
<td>2.28</td>
</tr>
<tr>
<td>Azelnidipine</td>
<td>16(^b)</td>
<td>48.3</td>
<td>0.0829</td>
<td>0.320</td>
<td>0.095</td>
<td>0.0304</td>
<td>0.0181</td>
<td>0.373</td>
<td>2.68</td>
</tr>
</tbody>
</table>

\(a\) Single administration.

\(b\) Repeated administration.

1,4-Dihydropyridine calcium antagonists are used worldwide in combination with other drugs. Many 1,4-dihydropyridine calcium antagonists inhibit CYP isozymes.\(^{22}\) Therefore, it is important to be able to accurately predict potential quantitative changes in the pharmacokinetics of drugs caused by co-administered calcium antagonists in order to avoid serious drug-drug interactions.\(^{22}\) In our study, the predicted AUC(\(\text{simvastatin}\))/AUC of azelnidipine (1.72) was similar to the observed clinical value (1.9)\(^{26}\) which, therefore, gives us confidence that the prediction of the simvastatin AUC(\(\text{simvastatin}\))/AUC (1.01) with benidipine might accurately reflect the clinical effect.

There are few reports of serious adverse effects occurring in clinical practice with concomitant usage of simvastatin and dihydropyridine calcium channel blockers. However, it is reported that rhabdomyolysis occurred in a patient taking a combination of simvastatin and diltiazem, a benzothiazepine calcium channel blocker.\(^{23}\) A clinical report suggested that the combined treatment of simvastatin and diltiazem caused a 5-fold increase in the AUC of simvastatin.\(^{26}\) Diltiazem and its major metabolite inhibit CYP3A4 in vitro.\(^{25}\) These results suggested that the CYP3A4-mediated simvastatin-diltiazem drug interaction resulted in the rhabdomyolysis. Hypercholesteremia is often accompanied by hypertension with an associated risk factor for coronary artery disease;\(^{26,27}\) therefore, simvastatin is often prescribed together with calcium channel blockers, including benidipine and azelnidipine. Our data predict that the AUC of simvastatin would not be increased by co-administration with benidipine and suggest that interactions for simvastatin based on CYP3A4 inhibition are less likely to occur with benidipine than with azelnidipine.

In our study, we used human liver microsomes to predict the drug-drug interaction potential of benidipine. However, CYP3A4 is also present in human small intestine.\(^{20}\) It was reported that metabolism of simvastatin was inhibited by grapefruit juice via an effect on
CYP3A4 primarily in the small intestine. The possibility of simvastatin-benidipine (and simvastatin-azelnidipine) metabolic interactions occurring in the small intestine should also be considered. However, prediction methods for intestinal drug-drug interactions have not been established. In this study, the prediction was performed from only the data obtained by the experiments using human liver microsomes. Hochman et al. reported that simvastatin was not a substrate of P-glycoprotein (P-gp) and that P-gp was unlikely to play a significant role in drug interactions for simvastatin from the transport and inhibition studies. Therefore, the inhibition of P-gp mediated transport was not considered in the present study. However, the potential of inhibition of P-gp mediated transport should also be considered to predict the drug-drug interactions in general.

In conclusion, benidipine and azelnidipine inhibited the metabolism of simvastatin in human liver microsomes. The $K_i$ value of benidipine corrected for the drug adsorption to microsomes (0.846 μM) was higher than that of azelnidipine (0.018 μM). Predictions of in vivo drug-drug interactions based on in vitro data indicated that the AUC of simvastatin co-administered with 8 mg of benidipine would not be increased (AUC(+)/AUC(-) = 1.01). On the contrary, the predicted AUC of simvastatin co-administered with 8 mg of azelnidipine was 1.72 times higher than the AUC of simvastatin when administered alone which is in agreement with the clinically observed value (1.9). These data suggest that benidipine is unlikely to cause significant drug interactions in clinical practice by inhibiting CYP3A4 activity.

References
20) Uji, Y., Sugimoto, T., Kobayashi, S. and Kobayashi,


