Co-administration of Fluvastatin and CYP3A4 and CYP2C8 Inhibitors May Increase the Exposure to Fluvastatin in Carriers of CYP2C9 Genetic Variants

Yuji Mukai, a Masayuki Narita, a Erika Akiyama, a Kanami Ohashi, a Yasutaka Horiuchi, a Yuka Kato, a Takaki Toda, a, b Anders Rane, b and Nobuo Inotsume a

a Division of Clinical Pharmacology, Hokkaido Pharmaceutical University School of Pharmacy; 7-jo 15-chome, 4-1 Maeda, Teine, Sapporo 066–8590, Japan; and b Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital; Huddinge, SE-14186 Stockholm, Sweden.

Received February 16, 2017; accepted April 14, 2017

Regular Article

Fluvastatin, which is one of the hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins), is primarily metabolized by CYP2C9 and to a lesser extent by CYP3A4 and CYP2C8. Predictions of drug–drug interactions (DDI) are important for the safety of combination therapies with statins, in particular drugs that are metabolized by CYP3A4. Little information is available regarding drug interactions with fluvastatin. Since CYP2C9 is a polymorphic enzyme, we investigated the effect of DDI via CYP2C9, CYP3A4, and CYP2C8 on fluvastatin pharmacokinetics by using a validated prediction method in relation to CYP2C9 variants. The predicted increases in the area under the concentration–time curve (AUC) in carriers of the CYP2C9*3 variant would cause a 3.23- and 2.60-fold increase in the AUC ratios, respectively, when compared to that for the carriers with the CYP2C9*1/*1 taking fluvastatin alone. We also predicted the effect of telmisartan when coadministered with fluvastatin. Our prediction results showed that the interaction between telmisartan and fluvastatin via CYP enzymes were negligible in clinical situations.

Key words fluvastatin; drug–drug interaction; genetic polymorphism; in vitro–in vivo extrapolation

Fluvastatin is one of the hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) that is used to treat dyslipidemia. Although many of the statins are primarily metabolized by CYP3A4, fluvastatin is mainly metabolized by CYP2C9 and to a lesser extent by CYP3A4 and CYP2C8. Therefore, fluvastatin is considered to be a good therapeutic agent for patients who should be treated with CYP3A4 inhibitors including calcineurin inhibitors. It has been demonstrated that three major metabolites were generated in human liver microsomes via CYP metabolic pathways. We have reported that the formation of 5-hydroxyfluvastatin (M-2) and N-desisopropyl fluvastatin (M-5) were mainly catalyzed by CYP2C9, whereas 6-hydroxyfluvastatin (M-3) was primarily catalyzed by CYP3A4. Myotoxicity, which is a leading cause of statin discontinuation, has been suggested to be the direct consequence of a dose-dependent inhibition of the HMG-CoA reductase. A previous study reported that approximately 60% of cases with statin-related rhabdomyolysis were related to a statin drug–drug interaction (DDI). For several statins, Ohno et al. have successfully predicted the area under the concentration–time curve (AUC) increase ratios caused by different inhibitors of CYP3A4. Hisaka et al. classified the risk of co-administration of CYP3A4 inhibitors based on the predicted AUC increase ratios. However, little is known about the effect of DDI on fluvastatin pharmacokinetics.

To date, there have been 60 genetic variants reported for the polymorphic enzyme, CYP2C9 (http://www.cypalleles.ki.se/cyp2c9.htm; accessed December 26, 2016). CYP2C9*2 and CYP2C9*3 are important polymorphisms, which result in reduction of CYP2C9 activity and expression level of CYP2C9 protein. Both variants have significantly higher frequencies in Caucasian populations compared with African and Asian populations. Kirchheiner et al. have reported that the AUC for 3R,5S-fluvastatin, which is the active enantiomer, was 3.1-fold higher in homozygous carriers of the CYP2C9*3 allele variant versus homozygous carriers of CYP2C9*1. Another study showed that the CYP2C9 polymorphism was related to the risk for fluvastatin-induced adverse drug reactions (ADRs). In addition, this study also found an increased risk of ADRs when carriers of CYP2C9 variant alleles were given CYP2C9 inhibiting medicines concomitantly. A subsequent case report found that an elevation of the muscle tissue biomarker creatine kinase in a Caucasian patient genotyped as CYP2C9*1/*3 appeared to be caused by a DDI between fluvastatin and telmisartan, and a normalization was noticed after switching from telmisartan to candesartan. Telmisartan has been shown to be an inhibitor of CYP2C9*4 and CYP2C8, while candesartan is an inhibitor of CYP2C9. Recently, DDI prediction methods have been reported that focus on differential AUC increase ratios related to CYP genotype and metabolism by multiple CYP isoforms. The aim of the present study was to predict the increase in AUC ratios for fluvastatin caused by DDI in the metabolic pathways of CYP2C9, CYP3A4, and/or CYP2C8, in carriers of different CYP2C9 allele variants compared to carriers of CYP2C9 wild-type.
MATERIALS AND METHODS

Chemicals Telmisartan and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Paclitaxel and fluvastatin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Irbesartan, which was used as an internal standard (IS) for the determination of the fluvastatin metabolites, and candesartan were obtained from Astatech (Bristol, PA, U.S.A.). Docetaxel trihydrate, which was used as an IS for the determination of 6α-hydroxypaclitaxel, and 6α-hydroxypaclitaxel were purchased from Toronto Research Chemicals (Toronto, Canada) and Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.), respectively. Supersomes™ from insect cells that expressed recombinant human CYP2C9 (rCYP2C9)*1 (lot: 414001), rCYP2C9*2 (lot: 5056006), rCYP2C9*3 (lot: 4203003), and control microsome (lot: 5217001), along with the 150-donor pooled human liver microsomes (pooled HLMs, lot: 38291) and monoclonal antibody for human CYP2C8 (MAB-2C8) derived from mouse were all obtained from Corning (Woburn, MA, U.S.A.). Metabolites of fluvastatin (M-2, M-3, and M-5) were kindly provided by Novartis Pharma (Tokyo, Japan). Other reagents and solvents were of either HPLC or special grades.

Estimation of the Effect of the Contribution Ratios (CRs) and the Fraction of the Activity of the CYP2C9 Mutated Allele Relative to the Wild-Type Allele (FA2C9) on Fluvastatin Metabolism Estimations of the effects of the CRs of CYP2C9 (CR2C9) and CYP3A4 (CR3A4) on the fluvastatin metabolism were determined in accordance with the method already validated, using data derived from two in vivo interaction studies. Briefly, various concentrations of fluvastatin were used to estimate the maximum velocity (Vmax) and Michaelis constant (Km) in relation to each metabolite formation. Protein concentration in an incubation mixture was adjusted with insect cell control microsome to yield a final concentration of 0.5 mg protein/mL. Fluvarustin concentrations were set at 0.5, 0.75, 1.0, 2.5, 5.0, 10, and 25 μM. Incubation time was set at 10 min for rCYP2C9*3 and 15 min for the others. Metabolites of fluvastatin were determined using the HPLC method described above. The Vmax and Km were estimated by the nonlinear least-squares fitting method of the GraphPad Prism software version 5.0 (GraphPad Software Inc.; San Diego, CA, U.S.A.). The intrinsic clearance (CLint) was then calculated using these parameters after correction with an intersystem extrapolation factor (ISEF), microsomal protein per gram of liver (MPPGL), and liver weight by the following equation:
\[ CL_{int} = \left\{ \frac{I_{SEF} \times V_{max}(rCYP2C9) \times CYP2C9 \text{ abundance}}{K_m(rCYP2C9)} \right\} \times \text{MPPGL} \times \text{liver weight} \tag{2} \]

where \( V_{max}(rCYP2C9) \) and \( K_m(rCYP2C9) \) are the \( V_{max} \) and \( K_m \) towards fluvastatin metabolism in \( rCYP2C9 \), respectively. Since we used \( rCYP2C9 \) derived from baculovirus expression system, the \( CL_{int} \) was corrected with the CLSEF value of 2.64 as described by Proctor et al.\(^{24} \) CYP2C9 abundance was used of 61 pmol/mg protein that was reported in a meta-analysis.\(^{25} \) The values of 34 mg protein/g of liver and 1603 g were used as MPPGL and liver weight, respectively.\(^{26,27} \)

Assuming that there are \( m \) categories of alleles and \( n_i \) alleles in each category, we estimated the \( F_{Ai} \) for the various CYP2C9 genotypes by using the following equation\(^{18} \):

\[ FA_i = \frac{1}{2} \sum_{i=1}^{m} n_i \cdot FA_i \tag{3} \]

where \( FA_i \) is the fraction of activity on fluvastatin metabolism, corrected \( CL_{int} \) of the variant CYP2C9 allele relative to the wild-type CYP2C9 allele. The reference value of the \( F_{A2C9} \) in the carriers who had the homozygote wild-type CYP2C9 was equal to 1.\(^{18} \)

**Prediction of the Increase in the AUC of Fluvastatin Caused by the CYP2C9 Genetic Variants** To predict the increase in the \( AUC \) of fluvastatin in carriers with mutated CYP2C9 allele versus in the patients homozygous for CYP2C9*1 at the same dosage (\( AUC^{XM}/AUC^{EM} \)), we used the following validated equation\(^{28,29} \):

\[ \frac{AUC^{XM}}{AUC^{EM}} = \frac{1}{1 - CR_{CYP2C9} \times (1 - FA_{2C9})} \tag{4} \]

where \( XM \) and \( EM \) represent the parameters in the carriers with the mutated CYP2C9 allele and the wild-type CYP2C9 allele, respectively. Predicted values of the increase in the \( AUC \) were then compared with the observed 3R, 3S-fluvastatin \( AUC \) values\(^{10} \) in carrier of CYP2C9 genetic variants. According to previous literatures,\(^{18,19} \) predicted values that were 50–200% of observed ratios were considered correct.

**Prediction of the Increase in the AUC of Fluvastatin in Carriers with the CYP2C9 Genetic Variants Caused by DDI Compared to That Observed in CYP2C9 Wild-Type Carriers Taking Fluvastatin Alone** The increase of the \( AUC \) of fluvastatin in the carriers of mutated CYP2C9 alleles caused by DDI (\( AUC^{XM}/AUC^{EM} \)) was predicted by using the following validated equation:

\[ \frac{AUC^{XM}}{AUC^{EM}} = 1/ \left\{ CR_{CYP2C9} \cdot FA_{2C9} \cdot (1 - I_{2C9}) + CR_{3A4} \cdot (1 - I_{3A4}) + CR_{3C8} \cdot (1 - I_{3C8}) + (1 - CR_{2C9} - CR_{3A4} - CR_{3C8}) \right\} \tag{5} \]

where \( AUC^{XM} \) is the \( AUC \) of fluvastatin in carriers with the mutated CYP2C9 allele when fluvastatin is administered with CYP inhibitors. \( AUC^{EM} \) is the \( AUC \) of fluvastatin in carriers with the CYP2C9 wild-type taking fluvastatin alone.

Owing to the lack of information about \( in vitro \) interaction studies that telmisartan and candesartan were used as CYP inhibitors, we estimated the \( I_X \) of these drugs were estimated using \( in vitro \) data and the following equation\(^{18,19} \):

\[ IX = \frac{[I]}{[I] + K_i} \tag{6} \]

where \([I]\) is the concentration of the inhibitor in the liver, which is usually the unbound concentration of inhibitor. However, since telmisartan is a substrate of the organic anion transporter (OATP) IB1,\(^{29} \) there is a high accumulation in the human liver after oral administration. This indicates that predictions of the \( IX \)s for telmisartan when using the unbound concentration would cause a false negative result, as predictions based on the unbound concentration of the inhibitor assume that the inhibitor distributes into the liver solely by passive diffusion. Candesartan does not accumulate in the human liver, which indicates that its distribution in the liver occurs mainly by passive diffusion. Therefore, in order to avoid any false negative results, we calculated the \( IX \)s of telmisartan with the maximum total hepatic inlet concentration ([\( I_{in} \)]) and candesartan with the maximum total concentration in the blood ([\( C_{max,blood} \)]\(^{30} \)) and was calculated by the following equation:

\[ [I]_{in} = C_{max,blood} + \frac{D \times k_a \times F_a}{Q_b} \tag{7} \]

where \( C_{max,blood} \) and \( D \) represent the maximum total concentration in the blood and the dosage of telmisartan, respectively. \( Q_b \) is the hepatic blood flow, \( k_a \) is the first-order absorption rate constant, and \( F_a \) is the fraction of the oral dose absorbed. Values of 1610 mL/min, 0.1 min\(^{-1} \), and 1.0 were used for the \( Q_b \), \( k_a \), and \( F_a \), respectively.\(^{30} \) The unbound concentrations of \([I]_{in} \) ([\( I_{in,a} \)]) were also calculated by multiplying \([I]_{in} \) and the plasma protein unbound fraction of telmisartan, 0.005.\(^{31} \)

The maximum concentration of the candesartan in the plasma after multiple oral administrations at the maximum clinical dose of 16 mg/d has been reported to be 0.264 µM.\(^{32} \) Since candesartan does not appear to distribute to blood cells,\(^{33} \) we used 0.55 as the smallest blood to plasma concentration ratio (Rb). Thus, the highest \( C_{max,blood} \) calculated was 0.145 µM. A previous study has examined the maximum concentrations of telmisartan that occurred in the plasma when using various dosages for multiple oral administrations.\(^{34} \) Furthermore the \( R_b \) of telmisartan has been reported to be 0.8.\(^{35} \) The \( C_{max,blood} \) values for the various dosages of telmisartan were calculated by multiplying the \( R_b \) and the corresponding plasma concentrations.\(^{34} \) Next, the \([I]_{in,a} \) and \([I]_{in} \) for the various dosages and the \( IX_{2C9} \) were calculated using Eqs. 6 and 7 (Supplementary Tables 1, 2).

The \( K_i \) values of telmisartan and candesartan for CYP2C9 were estimated from previous \( in vitro \) inhibition experiments.\(^{34,35} \) The authors reported the half maximal inhibitory concentration (IC\(_{50}\)) values calculated from incubation experiments under the condition that the substrates concentrations equaled to those \( K_m \). Therefore, we calculated their \( K_i \) values using the following equation:\(^{36} \):

\[ K_i = IC_{50} / 2 \tag{8} \]

we determined the \( K_i \) values of telmisartan for CYP2C9 to be 21.0\(^{40} \) and 2.39 µM\(^{35} \), respectively. We then used the mean value, 11.7 µM, for our further analysis of telmisartan. The IC\(_{50}\) value of candesartan for CYP2C9 has been reported to be 57.7 µM.\(^{40} \) and the \( K_i \) value for CYP2C9 was determined to
Table 1. Enzyme Kinetic Parameters for the Fluvastatin Metabolism by rCYP2C9s

<table>
<thead>
<tr>
<th>Enzyme kinetic parameters</th>
<th>rCYP2C9*1</th>
<th>rCYP2C9*2</th>
<th>rCYP2C9*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{max}$ (fmol/pmol P450/min) [95% CI]</td>
<td>273.6 [256.6–290.7]</td>
<td>271.3 [259.8–286.5]</td>
<td>193.4 [169.8–216.9]</td>
</tr>
<tr>
<td>$K_m$ (µM) [95% CI]</td>
<td>1.1 [0.87–1.4]</td>
<td>1.9 [1.6–2.2]</td>
<td>4.1 [2.7–5.5]</td>
</tr>
<tr>
<td>$CL_{int}$ (mL/pmol P450/min)</td>
<td>248.7</td>
<td>143.7</td>
<td>47.2</td>
</tr>
<tr>
<td>Corrected $CL_{int}$ (L/h)</td>
<td>131.0</td>
<td>75.7</td>
<td>24.8</td>
</tr>
</tbody>
</table>

Each parameter was estimated by using the nonlinear least-squares method and the means of the three metabolites formation determined from triplicate incubations. 95% CI: 95% confidence interval.

Table 2. Estimates of the Fraction of the Activity of the Mutated CYP2C9 Alleles Relative to the CYP2C9 Wild-Type Allele ($FA_{2C9}$) Obtained in the Current Study and Previous Meta-Analyses

<table>
<thead>
<tr>
<th>CYP2C9 genotypes</th>
<th>FA&lt;sub&gt;2C9&lt;/sub&gt;</th>
<th>Current study&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Castellan et al.&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dickinson et al.&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1/*2</td>
<td>0.79</td>
<td>0.82</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*1/*3</td>
<td>0.60</td>
<td>0.56</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*2/*2</td>
<td>0.58</td>
<td>0.70</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*2/*3</td>
<td>0.39</td>
<td>0.39</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>CYP2C9*3/*3</td>
<td>0.19</td>
<td>0.13</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Each $FA_{2C9}$ value was estimated by Eqs. 2 and 3. <sup>b</sup> Each $FA_{2C9}$ value was estimated with the data derived from in vivo pharmacokinetic studies. <sup>c</sup> Each $FA_{2C9}$ value was estimated with the data derived from in vitro yeast expressing system.

be 28.9 µM.

After we carried out an in vitro inhibition experiment of paclitaxel with pooled HLMs by using telmisartan and candesartan to determine the IC<sub>50</sub> values for CYP2C8, we then calculated the $K_m$ values for CYP2C8 using Eq. 8. The inhibition experiment was performed according to our previous report<sup>77</sup> with slight modification. HLMs (1.0 mg protein/mL) were incubated with inhibitors, paclitaxel, and NADPH (1 mM) in Tris–HCl buffer (50 mM, pH 7.4) in a total volume of 250 µL. Both of the inhibitor concentrations were set at 0, 5, 10, 50, 100, 500 µM, while the paclitaxel concentration was set at 7.5 µM, which was equal to the $K_m$. After incubation for 30 min, the reaction was stopped by adding 1 mL of ice-cold acetonitrile containing 1 µg/mL of docetaxel (15). The concentration of 6α-hydroxy-paclitaxel produced in each of the incubation mixtures was determined by an HPLC-UV method that has been previously described.<sup>37</sup> Mobile phase was delivered isocratically with 20 mM ammonium acetate buffer (pH 5.0) and acetonitrile (58:42, v/v) at a flow rate of 0.9 mL/min. The detection wavelength was set at 240 nm. The limit of quantification for 6α-hydroxy-paclitaxel was 100 ng/mL (115 nm). All incubations were carried out within the linear range of paclitaxel metabolism with respect to the protein concentration, incubation time and substrate concentration. IC<sub>50</sub> values were calculated using the nonlinear least-squares fitting method of the GraphPad Prism software. All incubations were performed in triplicate.

RESULTS

Estimation of the CR<sub>2C8</sub> and FA<sub>2C9</sub> on the Fluvastatin Metabolism The overall metabolic rate of fluvastatin in HLMs with MAB-2C8 and normal mouse IgG were 168.7 ± 3.6 and 186.6 ± 4.4 pmol/mg protein/min, respectively (mean ± standard deviation (S.D.), n=3). MAB-2C8 significantly inhibited the overall metabolism of fluvastatin by 10% ($p=0.0054$ by Student’s t-test), with the CR<sub>2C8</sub> for the fluvastatin metabolism estimated to be 0.10.

The estimated enzyme kinetic parameters and $FA_{2C9}$ values from our experiments that used rCYP2C9s are shown in Tables 1, 2, respectively. CYP2C9*2 caused an increase in the $K_m$ value for all of the fluvastatin metabolites, which resulted in a 42% decrease of the $CL_{int}$ compared to CYP2C9*1. CYP2C9*3 caused a decrease in the $V_{max}$ and an increase in the $K_m$, which resulted in an 81% decrease of the $CL_{int}$ compared to CYP2C9*1.

Prediction of the Increase of the AUC of Fluvastatin Caused by the CYP2C9 Genetic Variants Table 3 shows the predictions for $AUC^{NM1}/AUC^{EM}$ of fluvastatin. These predicted values were compared to the observed values of 3R,5S-fluvastatin that were derived from a clinical study.<sup>11</sup> Our predicted values were within a range of 50–200% of the previously determined values (Supplementary Fig. 1).

Prediction of the Increase of the AUC of Fluvastatin in Carriers with the CYP2C9 Genetic Variants Caused by DDI Compared to That Observed in CYP2C9 Wild-Type Carriers Taking Fluvastatin Alone Figure 1 shows the predictions for the $AUC^{NM7}/AUC^{EM}$ of fluvastatin. Although the CR<sub>2C8</sub> and CR<sub>2C9</sub> were much smaller than the CR<sub>2C8</sub> in the carriers with CYP2C9*1/*1, these results did indicate that potent CYP3A4 and CYP2C8 inhibitors might be able to cause 3.23-fold and 2.60-fold increases in the AUC of fluvastatin in the CYP2C9*1/*3 carriers, respectively, compared to the AUC<sup>EM</sup>.

Even if the highest C<sub>90</sub> blood was used, the IX<sub>2C8</sub> of candesartan was only estimated to be 0.01. Based on these results, we considered the inhibition of CYP2C9 by candesartan in clinical situations to be negligible.

Calculated concentration and the formation rate of 6α-hydroxy-paclitaxel in pooled HLMs in the absence of inhibitors were 958 ± 62.1 nm and 31.9 ± 2.1 pmol/mg protein/min, respectively (mean ± S.D., n=3). IC<sub>50</sub> values of telmisartan...
and candesartan for paclitaxel 6α-hydroxylation catalyzed by CYP2C8 were estimated to be 46.0 and 176.7 µM, and the $K_i$ values for CYP2C8 were estimated to be 23.0 and 88.4 µM, respectively. The $IX_{2C8}$ of telmisartan for the various dosages were calculated using Eq. 6, whereas the $IX_{2C8}$ of candesartan was estimated to be 0.00 (not measurable) even if the highest $C_{max,blood}$ was used. Based on these results, we believe the inhibition of CYP2C8 by candesartan in clinical situations to be negligible.

Table 4 shows the predicted $AUC_{XM}^{NM}/AUC_{EM}$ of fluvastatin
after coadministration of various dosages of telmisartan.

DISCUSSION

Results of the present study show the importance of the CYP2C9 genetic variants on the fluvastatin pharmacokinetics. We estimated CR2C9 and CR3A4 to fluvastatin metabolism from two different clinical studies,11,21,22 while CR2C8 from our in vitro experiment using MAB-2C8. Results indicated that MAB-2C8 only inhibited the M-3 formation (data not shown), which was consistent with our previous report and also that have been reported for the product labeling (Lescol®) in the U.S.A.38 Assuming that CYP2C9 genotypes only affect the activity of CYP2C9, activities of CYP2C8 and CYP3A4 would conserve among carriers of all CYP2C9 genotypes. CR2C9 in carriers of CYP2C9*2 and CYP2C9*3 variants would be lower relative to those with CYP2C9 wild-type, indicating that the metabolic pathways by CYP2C8 and CYP3A4 would be more important than CYP2C9 on fluvastatin metabolism in carriers of CYP2C9 variants. This assumption is consistent with our result (Fig. 1A) and the previous result reported by Kumar et al.,39 where the inhibitory effects of CYP2C9 inhibitors decreased in carriers of CYP2C9*1/*3 and CYP2C9*3/*3 compared to the wild-type carriers.

The FA2C9 of the 6 diplootypes of CYP2C9 that were calculated in this study were in a good agreement with them estimated in meta-analyses with in vitro data derived from yeast expressing system50 and in vivo data39 (Table 2). Although the values obtained in our current study were calculated based on an in vitro experiment with a single lot of rCYP2C9 proteins for each CYP2C9 variant that did not distinguish the concentrations for each of the fluvastatin enantiomers, the AUC SM/AUC EM of fluvastatin predicted when using Eq. 4 were within a range of 50–200% of the observed AUC SM/AUC EM of the active enantiomer, 3R,5S-fluvastatin, that was derived from a previous clinical study39 (Table 3). Thus, these results indicate that the predicted AUC SM/AUC EM of fluvastatin in this study correspond to the values of 3R,5S-fluvastatin and that in vitro prediction of the in vivo situation is reasonably good.

Muscle toxicity, which is one of the leading causes of statin discontinuation, has been suggested to be dose-dependent.5 Based on our predictions, the AUC of fluvastatin in the carriers of CYP2C9*3/*3 variant was estimated to be 2.1-fold higher versus the carriers with the CYP2C9*1/*1 at the same dosage (Table 3). Hisaka et al. initially proposed the Pharmacokinetic Interaction Significance Classification System (PISCS).8 According to this classification system, the combinations of the CYP3A4 inhibitors and the statins that are primarily metabolized by CYP3A4 caused a >7-fold and >2-fold increase of AUC were categorized as ‘contra-indication’ and ‘warning/caution,’ respectively. The predicted AUC SM/AUC EM in the carriers with the CYP2C9*3/*3 variant was greater than the boundary for the ‘warning/cautions’ (Table 3), this indicates that further decrease of fluvastatin clearance such as DDI in those patients might increase the risk of ADRs of fluvastatin even though the effect of DDI is negligible in population of CYP2C9*1/*1 carriers. In fact, Mirošević Skvrc et al. have found an increased risk of ADRs when carriers of CYP2C9 variant alleles were given CYP2C9 inhibiting medi-
Ohno et al.⁷ and Hisaka et al.⁸ have reported that careful attention needs to be paid to the co-administration of CYP3A4 inhibitors when using statins that are mainly metabolized by CYP3A4. It has also been reported that fluvastatin should be considered to be a good choice for patients who need to be treated by CYP3A4 inhibitors such as calcineurin inhibitors. Our prediction indicated that co-administration of a potent CYP3A4 inhibitor with an \(I_{2C9}\) of 1.0 in patients genotyped as \(CYP2C9*1/*3\) would see a 3.2-fold increase in the \(\frac{AUC_{\text{est}}}{AUC_{\text{in}}}\) (Fig. 1B). Although negligible interaction has been reported between fluvastatin and itraconazole in healthy volunteers, in which the information about CYP2C9 genotype was not available, our results do suggest that co-administration of potent CYP3A4 inhibitors would increase the risk of fluvastatin-induced ADRs in homozygous carriers of \(CYP2C9*3\). This finding can be explained by a relative increase in \(C_{\text{max, blood}}\) along with decreasing \(C_{\text{R,3A4}}\) to fluvastatin metabolism in carriers of that mutative allele relative to the wild-type carriers, as discussed above. To date, 60 genetic variants for CYP2C9 have been reported, while our current study only used 6 diplotypes. Therefore, a further study will need to be undertaken in order to definitively determine the effect of other CYP2C9 genetic variants on the fluvastatin pharmacokinetics.

To investigate the effect of telmisartan and candesartan on the fluvastatin pharmacokinetics, we calculated the \(I_{2C9}\) and \(I_{2C29}\) for both of the drugs. Although candesartan showed inhibitory effects for CYP2C9 and CYP2C8, both of the IXs of candesartan were essentially zero, even when using the highest \(C_{\text{max, blood}}\) after multiple oral administrations for the calculation. Since telmisartan is a substrate of OATP1B3 ²⁹) the inhibitory effects were negligible when using \(\frac{AUC_{\text{est}}}{AUC_{\text{in}}}\) in,u for the prediction (Table 4A). Our results using \(\frac{AUC_{\text{est}}}{AUC_{\text{in}}}\) in,u for the prediction showed that when 160 mg of telmisartan was co-administered with fluvastatin in carriers of any mutated CYP2C9 alleles, the PISCs classified the results as ‘warning/caution’,³⁰) whereas the inhibitory effects were negligible when using \(\frac{AUC_{\text{est}}}{AUC_{\text{in}}}\) in,u for the prediction (Table 4B). A recent case report ¹³) has suggested that a pharmacokinetic interaction occurred between fluvastatin and telmisartan, but not candesartan, in a subject with the CYP2C9*1/*3 genotype who was taking 20 mg of telmisartan. Based on our predictions using the theoretically highest concentration, \(\frac{AUC_{\text{est}}}{AUC_{\text{in}}}\) for CYP2C9 \(\text{II}/\text{III}\) variants after the co-administration of 20 mg of telmisartan would be 1.51. It was slightly higher than the 1.35 that was predicted in the absence of inhibitors or in the presence of candesartan in subjects with the CYP2C9*1/*3 variants (Table 3). Furthermore, an in vivo interaction study showed only 1.09-fold increase in \(AUC\) of warfarin with the co-administration of 120 mg of telmisartan. ⁴¹) These findings indicate that it is unlikely to occur the interaction between telmisartan and fluvastatin via CYP enzymes in clinical situations. Since some OATPs and an ATP-binding cassette sub-family G member 2 (ABCG2) efflux transporter are associated with disposition of fluvastatin, ¹²,⁴²,⁴³) the interactions via those transporters between fluvastatin and telmisartan might occur in vivo situation. An additional study will need to be undertaken in order to further elucidate the DDI on the fluvastatin pharmacokinetics.

In summary, we found that CYP2C9 genotype affected DDI-potential of fluvastatin. The impact of CYP3A4 inhibitors would be stronger if the total clearance of fluvastatin to a greater extent depended on CYP3A4 rather than CYP2C9 in patients with a poor metabolizer genotype CYP2C9*3/*3.

Acknowledgments The authors would like to thank Professor Erik Eliasson of the Karolinska Institutet for his valuable advice about this manuscript. This work was supported by a fund from an Education and Research Grant from the Hokkaido Pharmaceutical University School of Pharmacy.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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