DIFFERENTIAL INTERACTION OF 3-HYDROXY-3-METHYLGTRURYL-COA REDUCTASE INHIBITORS WITH ABCB1, ABCC2, AND OATP1B1

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ABSTRACT:

The present study examined the interaction of four 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (atorvastatin, lovastatin, simvastatin, and pravastatin) with multidrug resistance gene 1 (MRP2, ABCC2), the IC50 values for efflux transporters (MDR1, MRp2, and OATP1B1), and the IC50 values for the uptake transporter OATP1B1 were 3- to 7-fold lower for statins in the acid form compared with the corresponding lactone form. These data demonstrate that lactone and acid forms of statins exhibit differential substrate and inhibitor activities toward efflux and uptake transporters. The interconversion between the lactone and acid forms of most statins exists in the body and will potentially influence drug-transporter interactions, and may ultimately contribute to the differences in pharmacokinetic profiles observed between statins.

Statins decrease intracellular cholesterol biosynthesis by reversibly inhibiting the microsomal enzyme hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase. Most statins are given in the orally active β-hydroxy acid form, except lovastatin and simvastatin, which are administered as inactive lactone prodrugs (Corsini et al., 1999; Reinoso et al., 2001). Both lactone and acid forms were observed in the systemic circulation following oral administration of atorvastatin (Kantola et al., 1998a), lovastatin (Neuvonen and Jalava, 1996), simvastatin (Kantola et al., 1998b; Prueksaritanont et al., 2002b), and cerivastatin (Backman et al., 2002) in humans and/or animals, indicating that interconversion occurs between the lactone and acid forms of these statins. It has been proposed that statin lactones are hydrolyzed to their acid forms chemically or enzymatically by esterase and paraoxonases (Billecke et al., 2000; Draganov et al., 2000). The statin acids are converted to the corresponding lactones by the CoASH-dependent and/or the acyl glucuronide intermediate pathways (Prueksaritanont et al., 2002a). Additionally, statins such as atorvastatin, lovastatin, simvastatin, and pravastatin are metabolized mainly by CYP3A4 in humans (Corsini et al., 1999). Therefore, inhibition of CYP3A4-mediated metabolism has been one of the major causes of drug interaction of these statins (Reinoso et al., 2001), except for pravastatin, which undergoes degradation under the acidic conditions of the stomach, as well as presystemic metabolism (Hatanaka, 2000). Both the acid and lactone forms of atorvastatin, simvastatin, and lovastatin have been reported to be substrates and inhibitors of CYP3A4, with the lactone forms showing stronger inhibitory effect on CYP3A4-mediated metabolism of mexazolam, compared with the corresponding acid forms (Ishigami et al., 2001), suggesting that the increased lipophilicity of lactone forms of these statins renders stronger inhibition of CYP3A4-mediated metabolism.

The lactone and acid forms of statins also exhibit different inhibitory effects on P-glycoprotein (ABCB1)-mediated transport of rhodamine 123 (Bogman et al., 2001), with the lactone forms of atorvastatin, simvastatin, and lovastatin exhibiting more potent P-glycoprotein inhibition than their corresponding acid forms. P-glycoprotein, present in most tissues, including liver, intestine, kidney, and brain, has been shown to limit the oral absorption and brain penetration of many drugs (Chen and Pollack, 1999; Boyd et al., 2000; Chen et al., 2003a,b). Although it is not surprising that some of the statins, such as atorvastatin, have been shown to be substrates of P-glycoprotein (Boyd et al., 2000; Wu et al., 2000), considering the overlapping substrate specificity of CYP3A4 and P-glycoprotein (Zhang et al., 1998; Hall et al., 1999), it is not known whether the lactone and acid forms of statins have different affinities as P-glycoprotein substrates.

ABBREVIATIONS: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ABC, ATP-binding cassette; MDCK, Madin-Darby canine kidney; OATP, organic anion-transporting polypeptide; MRP2, multidrug resistance-associated protein 2; MDR1, multidrug resistance gene 1; PBS, phosphate-buffered saline; AM, acetoxymethyl ester; DMSO, dimethyl sulfoxide A; apical; B, basolateral.
Similar to P-glycoprotein, multidrug resistance-associated protein 2 (MRP2, ABCB2) is an efflux transporter and expressed in the intestine and liver (Borst et al., 1999; Mottino et al., 2000), and it has been shown to limit the oral absorption and mediate the biliary excretion of drugs, including statins (Yamazaki et al., 1997; Chen et al., 2003c). Biliary excretion of pravastatin was impaired significantly in MRP2-deficient Eisai hyperbilirubinemic rats (EHBRRs) compared with normal rats, suggesting that MRP2 mediates the biliary excretion of pravastatin. Furthermore, pravastatin (Hsiang et al., 1999) and cerivastatin (Shitara et al., 2003) have been shown to be substrates of organic anion-transporting polypeptide 1B1 (OATP1B1, SLC21A6) and OATP2 (SLC21A2), two main liver-specific uptake transporters (Hsiang et al., 1999; Hagenbuch and Meier, 2004). More recently, two elegant studies (Shitara et al., 2003, 2004) examined the relative contributions of metabolism versus transport in the clinically observed interaction between cyclosporin A and cerivastatin (Muck et al., 1999) and between gemfibrozil and cerivastatin (Backman et al., 2002). The results indicated that the increase in cerivastatin systemic concentrations with cyclosporin A is likely due to the inhibition of the hepatic uptake transporter OATP1B1 rather than inhibition of CYP3A4- or CYP2C8-mediated metabolism, whereas interaction with gemfibrozil is due to a combination of inhibition of OATP1B1-mediated hepatic uptake and CYP2C8-mediated metabolism.

Data to date suggest that, apart from metabolism-based interactions, transport interactions can modify the pharmacokinetics and, consequently, the safety profiles of statins and coadministered drugs. It is not known whether statins as a class interact with OATP1B1 and MRP2, and whether the lactone and acid forms of statins differ in their interaction with these transporters.

The present study investigated the interaction of four model HMG-CoA reductase inhibitors (atorvastatin, lovastatin, and simvastatin in both acid and lactone forms, and pravastatin in acid form only) with P-glycoprotein, MRP2, and OATP1B1 in vitro, to understand the potential contributions of these transporters to the different pharmacokinetic properties of statins.

Materials and Methods

Materials. Atorvastatin, in both acid and lactone forms, was synthesized at Pfizer (Ann Arbor, MI). Lovastatin, simvastatin, pravastatin, and verapamil HCl were purchased from Sigma-Aldrich (St. Louis, MO). The acid form of lovastatin and simvastatin was prepared by hydrolysis of the corresponding lactone under alkaline condition. All the other chemicals and reagents were the highest grade available from commercial sources. Structures and physical-chemical parameters of the statins used in the present study are listed in Table 1.

Transcellular Transport Studies across Cultured Cell Monolayers. The transport studies method was a slight modification from Smith et al. (2001). Briefly, Madin-Darby canine kidney (MDCK) II and MDCKII-MDR1 cells expressing human MDR1 gene (P. Borst, Netherlands Cancer Institute, Amsterdam, The Netherlands) were seeded onto the membrane of the HTS 96-multicell inserts obtained from Falcon; BD Biosciences Discovery Labware (Bedford, MA). The inserts containing seeded cells were then placed into prefilled BD Biosciences Discovery Labware feeding trays filled with 37 ml of minimal essential medium alpha growth medium. Cells were incubated at 37°C for 5 h. The medium was removed and assayed using a high-speed high-pressure liquid chromatography-mass spectrometry analysis as described by Brockman et al. (2000). The extent of permeation (Papp) values were calculated for both A-B and B-A transport. The ratio of Papp values between B-A and A-B, after correcting for the efflux in the parental MDCKII cells, was defined as the efflux ratio and represents human P-glycoprotein-mediated efflux (Chen et al., 2003a).

Stimulation of ATP Hydrolysis. The ability of compounds to stimulate ATP hydrolysis was examined using the membrane vesicle-expressing human P-glycoprotein (BD Gentest, Woburn, MA). The method used to determine the drug-simulated ATPase activity was optimized based on the manufacturer’s protocol. Briefly, membranes (8 μg) were incubated at 37°C for 5 min in 60 μl of medium consisting of 50 mM 2-morpholinoethanesulfonic acid, 50 mM KCl, 2 mM diethiothreitol, 2 mM ethylene-glycol-bis-2-aminoethylether-tetraacetic acid, 2 mM Tris-HCl, and 5 mM Na2HPO4 (pH 6.8) in the presence of test compounds. The reaction was initiated by the addition of 20 μl of 3 mM ATP (magnesium salt) and was stopped 20 min later by the addition of 30 μl of 10% sodium dodecyl sulfate. Detection reagent (180 μl of solution containing 7 mM ammonium molybdate, 3 mM zinc acetate, and 16% ascorbic acid, pH 5.0) was added to all wells and incubated at 37°C for 20 min. The absorbance at 850 nm was measured using a plate reader (Safire; Tecxan, Maennedorf, Switzerland). The drug-stimulated ATPase activity (nmol/min/mg protein) was determined as the difference between the amounts of inorganic phosphate released from ATP in the absence and presence of vanadate (100 μM). Potassium phosphate standards were prepared in each plate, and verapamil served as the positive control. Kinetic parameters (apparent Km, and Vmax) were estimated by fitting of the drug concentrations and ATP hydrolysis activity into the Michaelis-Menten equation.

Inhibition of MDR1-, MRP2-, and OATP1B1-Mediated Efflux of Calcein-AM. Parental MDCK and three additional MDCK cell lines transfected with human MDR1 and MRP2 (P. Borst, Netherlands Cancer Institute) and rat MRP2 (D. Keppler, University of Heidelberg, Heidelberg, Germany) were grown and maintained in minimal essential medium supplemented with 10% fetal bovine serum. Cells were incubated at 37°C with 5% CO2 for 24 h before the study. For the studies, each cell line (50,000 cells per well) was plated into Costar 3904 black 96-well plates (PerkinElmer Life and Analytical Sciences, Boston, MA) with 100 μl of medium supplemented with 1% fetal bovine serum, and allowed to become confluent overnight. Test compounds were added to monolayers in 100 μl of culture medium containing 1% DMSO as solvent. Plates were incubated at 37°C for 30 min. Calcein-AM (Molecular Probes, Eugene, OR) was added in 100 μl of phosphate-buffered saline (PBS) to yield a final concentration of 2.5 μM. Plates were incubated for another 30 min. Cells were then washed three times with ice-cold PBS. PBS was then added to the cells, and the cells were then read with a Victor2 fluorometer (PerkinElmer Life and Analytical Sciences) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. P-glycoprotein inhibition was calculated using the following equation: % Inhibition = [(The amount of efflux)untreated − (The amount of efflux)inhibited)/ (The amount of efflux)untreated] × 100. The “The amount of efflux” was defined as the fluorescence from MDCK cells subtracted by that from the MDR1-MDCK cells, and “The amount of inhibition” was defined as [(The amount of efflux)untreated − (The amount of efflux)inhibited)/ (The amount of efflux)untreated]. The IC50 values were determined by fitting the percentage of inhibition-concentration data into the Hill equation using the ordinary least squares method of the LabStats Excel Add-in (Nonclinical Statistics group, Pfizer Central Research, Sandwich, Kent, UK). When fitting the Hill equation, cytotoxic data points for some compounds at the highest test concentration were removed. The cytotoxic data points were apparent because of the loss of calcein fluorescence at the highest test concentration of some compounds. The Hill slope, and the minimum and maximum percentage inhibition were not fixed, and the percentage inhibition data were not weighted. The IC50 was based on the concentration at which the apparent 50% inhibition was achieved (i.e., apparent IC50). Vinblastine, a known inhibitor of MRP2/MPD and MDR1, was used as the positive control.

Inhibition of OATP1B1-Mediated Uptake of Estradiol 17β-D-Glucoconide. Human embryonic kidney (HEK 293) cells transfected with OATP1B1 were obtained from Professor D. Keppler (University of Heidelberg). [3H]Estradiol 17β-D-glucuronide (44 Ci/mmol, >97% purity) was obtained from PerkinElmer Life and Analytical Sciences. On day 1, ~2.5 × 104 HEK 293 cells, transfected with the cDNA for OATP1B1, were seeded on a 24-well poly-o-lysine-coated plate (BD BioCoat; BD Biosciences Discovery Labware).
and cultured overnight. On the following day, the medium was changed and the cells were cultured with 10 mM sodium butyrate for 24 h to increase the expression of OATP1B1. On day 3 (the day of the assay), the cells were first washed with uptake buffer (142 mM NaCl, 5 mM KCl, 1 mM KH₂PO₄, 1.2 mM MgSO₄, 1.5 mM CaCl₂, 5 mM glucose, and 12.5 mM HEPES, pH 7.3). Uptake was then initiated by the incubation of the cells with a solution (250 μl) containing [³H]estradiol 17β-D-glucuronide (0.02 μM) and the test compound (0–100 μM) in DMSO for 2 min. The DMSO in the final incubation solution was 1%. At the completion of the assay, uptake was stopped by washing the cells three times with ice-cold buffer. The cells were then lysed in 1% SDS and the accumulated radioactivity was determined. Each data point represented the mean and standard deviation of triplicates performed in parallel. Rifamycin SV, an OATP1B1 inhibitor (Tirona et al., 2003), was used as a positive control. Percentage activity was calculated by assuming the zero inhibitor (mean of three replicates) as 100% activity. The activity for each concentration was then reported as percentage activity remaining. The IC₅₀ values were estimated by the Hill equation and nonlinear regression in Origin (OriginLab Corp., Northampton, MA).

Results

Transcellular Transport Studies across Cultured Cell Monolayers. An initial experiment showed that transport was linear within a 5-h incubation time period, and degradation of the statins tested over the time course was <20% (data not shown). Therefore, 5 h was used as the duration for the subsequent studies. As shown in Table 2, for each pair of statins, the acid form displayed a slightly higher efflux ratio than did the corresponding lactone form. Overall, the statins (lactone and acid forms) were shown to be weak-to-moderate substrates of P-glycoprotein compared with the positive controls quini-
dine (good substrate) and prazosin (moderate substrate), which had
efflux ratios of 15 and 3.5, respectively. Simvastatin lactone did not
show any efflux in this transport assay, suggesting that simvastatin
lactone is not a P-glycoprotein substrate.

**Stimulation of ATP Hydrolysis.** The plots of ATP hydrolysis as a
function of drug concentrations for the seven statin compounds are
shown in Fig. 1. The estimated apparent kinetic parameters are listed
in Table 2. A significant stimulatory effect was exhibited by lovastatin
lactone, whereas lovastatin acid did not exhibit ATP hydrolysis.
Simvastatin lactone stimulated P-glycoprotein-mediated ATP hydro-
lysis at concentrations below 100 μM, whereas simvastatin acid did
not exhibit activity. At concentrations of 100 μM and 150 μM,
simvastatin lactone decreased ATPase activity. This may be evidence
of P-glycoprotein inhibition. Substrate-inhibitor mixed-function ki-
etics was also evident for atorvastatin lactone, which appeared to
activate P-glycoprotein ATP hydrolysis up to 5 μM and decreased
activity at higher concentrations. Atorvastatin acid and pravastatin

![Fig. 1. Concentration-dependent effect of statins on ATP hydrolysis by P-glycoprotein. Significant ATP hydrolysis data were fitted to the Michaelis-Menten equation to determine the apparent $K_m$ and $V_{max}$. Closed symbols represent data included in analysis. Data with open symbols were not used in analysis. Each point reflects the average of duplicate samples.](image-url)
acid did not significantly stimulate P-glycoprotein-mediated ATP hydrolysis at any of the concentrations tested.

**Inhibition of MDR1-, MRP2-, and Mrp2-Mediated Efflux of Calcein-AM.** The plots of percentage inhibition of MDR1-, MRP2-, and Mrp2-mediated uptake of calcein-AM as a function of concentrations of statins are shown in Figs. 2 to 4, respectively. The IC₅₀ values are shown in Table 3. For MDR1, atorvastatin, simvastatin, and lovastatin lactone forms showed an IC₅₀ value of ~10 µM, whereas the IC₅₀ value of the acid forms was >100 µM. Similarly, the lactone form of atorvastatin, lovastatin, and simvastatin exhibited more potent inhibitory effects on MRP2- and Mrp2-mediated calcein transport compared with their corresponding acid forms. Pravastatin (100 µM) did not inhibit MDR1-, MRP2-, or Mrp2-mediated transport of calcein. The IC₅₀ values of vinblastine toward MDR1 and MRP2/Mrp2 were 29 and 21 µM, respectively.

**Inhibition of OATP1B1-Mediated Uptake of Estradiol 17β-D-Glucuronide.** The plots of percentage inhibition of OATP1B1-mediated transport of estradiol 17β-D-glucuronide by various statins are shown in Fig. 5 with the IC₅₀ values in Table 3. The results showed that the acid form exhibited more potent inhibition toward OATP1B1 compared with its corresponding lactone form. However, pravastatin, an acid, did not inhibit OATP1B1-mediated uptake of estradiol 17β-D-glucuronide. In all cases, these statins were much less potent inhibitors of OATP1B1 compared with the positive control, rifamycin SV (IC₅₀ of 0.23 ± 0.07 µM).

**Discussion**

Several studies have examined the interaction of statins with P-glycoprotein. For example, simvastatin, lovastatin, and atorvastatin, but not pravastatin, inhibited both mouse mdr1a- and human MDR1-mediated transport of daunorubicin or rhodamine 123 (Wang et al., 2001). Bogman et al. (2001) confirmed that simvastatin, lovastatin, and atorvastatin were P-glycoprotein inhibitors, whereas pravastatin was not. These studies demonstrate that pravastatin, which is more hydrophilic than all other statins, differs in its inhibitory property toward P-glycoprotein-mediated transport, suggesting that a certain lipophilicity may be necessary for interaction with P-glycoprotein. It is not known whether this requirement holds true for interaction with other transporters such as MRP2/Mrp2 and OATP1B1, and whether lactone and acid forms of statins differ in these interactions. There-
fore, the objective of the present study was to examine the interaction of three pairs of statins, i.e., atorvastatin, lovastatin, and simvastatin, in both acid and lactone forms, and pravastatin with P-glycoprotein, MRP2/Mrp2, and OATP1B1.

The lactone form of all three statins exhibited more potent P-glycoprotein inhibition compared with their corresponding acid forms (Table 3). The IC₅₀ values were similar among the three lactones and up to 10-fold lower compared with their acid form. Pravastatin (acid form only), although much more hydrophilic compared with other three statins in the acid form, showed similar weak inhibition. Our data on the inhibition of P-glycoprotein-mediated transport by atorvastatin acid differs from what was observed by Bogman et al. (2001), who reported that atorvastatin acid was also a P-glycoprotein inhibitor. The discrepancy between the two studies could be due to the differences in the probe P-glycoprotein substrate (rhodamine 123 versus calcien AM) and the species of P-glycoprotein (mouse mdr1a/b versus human MDR1) used.

The major physical-chemical difference between the lactone and acid form for each statin is that the lactone is more basic and has a higher cLogP compared with the acid (Table 1). The fact that the lactone form showed stronger inhibition of P-glycoprotein-mediated transport suggests the importance of lipophilicity and basicity in P-glycoprotein inhibition. The stronger P-glycoprotein inhibition of the lactone compared with the acid form, and their coexistence in vivo (Kantola et al., 1998a,b; Backman et al., 2002; Prueksaritanont et al., 2002b), may also imply that the lactone form may have been the major contributor to the clinically observed drug interaction between statins and digoxin, a known P-glycoprotein substrate. Indeed, increased digoxin concentrations were observed with oral coadministration of atorvastatin (Boyd et al., 2000) and simvastatin (Stockley, 1999), but not with pravastatin (Stockley, 1999). Similar concentrations of the acid and lactone forms were observed following oral administration of atorvastatin (Kantola et al., 1998a) and simvastatin (Kantola et al., 1998b; Prueksaritanont et al., 2002b) in humans and animals. Although it is not known whether in vivo pravastatin lactone is similar to the acid form following oral administration of pravastatin, the combined in vitro and in vivo data suggest that pravastatin lactone is, at most, a weak P-glycoprotein inhibitor. This finding was true for the inhibition of CYP3A4-mediated metabolism of mexazolam, where pravastatin lactone showed a weak inhibition similar to that of ator-

![Inhibition of statins toward MRP2-mediated efflux of calcien-AM. The symbols are observed data, and the line represents the fit of the data with the Hill equation.](image-url)
TABLE 3

Inhibition of statins to MDR1, Mrp2, MRP2, and OATP1B1

Data are expressed as mean ± SD, n = 3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Unbound C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; MDR1</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; MRP2</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; Mrp2</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; OATP1B1</th>
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<td>Pravastatin acid</td>
<td>0.0648&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td>Atorvastatin acid</td>
<td>0.0024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.87 ± 0.23</td>
</tr>
<tr>
<td>Atorvastatin lactone</td>
<td>0.0024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14 ± 1</td>
<td>15 ± 3</td>
<td>15 ± 6</td>
<td>2.6 ± 1.4</td>
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<tr>
<td>Lovastatin acid</td>
<td>0.0024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;33</td>
<td>4.0 ± 1.2</td>
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<tr>
<td>Lovastatin lactone</td>
<td>0.0025&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10 ± 4</td>
<td>&gt;33</td>
<td>35 ± 3</td>
<td>28 ± 16</td>
</tr>
<tr>
<td>Simvastatin acid</td>
<td>0.0039&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>3.6 ± 1.7</td>
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<tr>
<td>Simvastatin lactone</td>
<td>0.0041&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 ± 8</td>
<td>25 ± 2</td>
<td>17 ± 2</td>
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<tr>
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<td>not applicable</td>
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<sup>a</sup> Based on a 40-mg oral dose. Adapted from data in Corsini et al. (1999).

<sup>b</sup> The concentration of lactone form was calculated based on the ratio of acid to lactone from other clinical studies (atorvastatin: Mazzu et al., 2000; Kantola et al., 1998a; lovastatin: Kyrklund et al., 2001; simvastatin: Kantola et al., 1998b).

<sup>c</sup> Positive control as an inhibitor of MDR1 and MRP/Mrp2 (mean of duplicate experiments).

<sup>d</sup> Positive control as an inhibitor of OATP1B1.

FIG. 4. Inhibition of the statins toward Mrp2-mediated efflux of calcein-AM. The symbols are observed data, and the line represents the fit of the data with the Hill equation.
vastatin and simvastatin acid (Ishigami et al., 2001). The log D value of pravastatin lactone was comparable to that of atorvastatin and simvastatin acid (Ishigami et al., 2001).

Interestingly, in the P-glycoprotein substrate assay using MDR1-MDCK cells, the lactone form of the statins showed a trend of lower efflux ratio compared with its corresponding acid form. However, the magnitude of the difference (less than 2-fold) in the efflux ratio is smaller compared with the difference (10-fold) in P-glycoprotein inhibition between the acid and lactone forms. Overall, except for simvastatin lactone, which is not a substrate of P-glycoprotein, the rest of the statin compounds (acid and lactone) tested are weak-to-moderate P-glycoprotein substrates. Atorvastatin acid is the only statin that has been examined and shown to be a P-glycoprotein substrate by other investigators (Wu et al., 2000).

Consistent with the results from the P-glycoprotein inhibitor assay, the ATPase assay showed that the lactone form had greater stimulatory effects on the ATPase activity compared with its acid form. Pravastatin did not activate the ATP hydrolysis, consistent with it being a very weak substrate and not an inhibitor of P-glycoprotein. However, no activation of ATP hydrolysis was observed for the other three statins in acid form, although MDR1-MDCK transport assay demonstrated that they were moderate P-glycoprotein substrates. The discrepancy between the two assays (transport and ATPase) could be partly due to the fact that, unlike the MDR1-MDCK assay, which measured transport of potential P-glycoprotein substrates, the ATPase assay is a generic readout on the release of inorganic phosphate and does not directly measure transport (Scarborough, 1995; Polli et al., 2001). Polli et al. (2001) showed that the ATPase assay could render a false-negative result for many drugs identified as P-glycoprotein substrates in the transport assay. Furthermore, the ATPase assay cannot distinguish a substrate from an inhibitor. Therefore, all statins in the lactone form, which were potent P-glycoprotein inhibitors, showed greater stimulatory effects compared with the acid form in the ATPase assay.

Although the present study has shown that all statins except simvastatin lactone are P-glycoprotein substrates, the likelihood of increasing the systemic concentration of statins and, consequently, significant drug interaction when statins are coadministered with a
OATP1B1-mediated transport of [3H]estradiol 17\textsubscript{a}tus may render a statin to be a more potent OATP1B1 inhibitor. Atorvastatin, lovastatin, and simvastatin. However, pravastatin acid, showed that the acid form was more potent than the lactone form for inhibition of OATP3A4-mediated metabolism, rather than the P-glycoprotein-mediated transport of atorvastatin at the gut level.

Similar to P-glycoprotein inhibition, the lactone form is at least 3- to 4-fold more potent than the acid form, based on the IC\textsubscript{50} values for MRP/Mrp2 inhibitors. Unfortunately, literature on the structure-requirement as MR2/Mrp2 inhibitors is scarce. Like P-glycoprotein, MRP2/Mrp2 is expressed in the intestine (Mottino et al., 2000) and biliary canaliculi (Borst et al., 1999), and can reduce the oral absorption and biliary excretion of MR2 substrates. MR2 is less important in limiting oral absorption than in reducing the biliary excretion of its substrates (Chen et al., 2003c). The clinical significance of MRP2 inhibition by statins warrants further studies.

In contrast to the observation of the inhibitory effects on P-glycoprotein and MR2/Mrp2 inhibitors, inhibition of OATPB1 by these statins showed that the acid form was more potent than the lactone form for atorvastatin, lovastatin, and simvastatin. However, pravastatin acid, which differs from the other three statin acids in its lipophilicity, failed to show any inhibition toward OATP1B1, suggesting that unlike P-glycoprotein, reduced lipophilicity and, possibly, acidic stigmas may render a statin to be a more potent OATP1B1 inhibitor. However, this may not apply to nonstatin drugs. Several nonanionic drugs including rifampin, cyclosporin A, indinavir, nelfinavir, saquinavir, and ritonavir have been shown to effectively inhibit OATPB1-mediated transport of \textsuperscript{1}H\textsubscript{1}estradiol 17\textsubscript{b}-glucuronide, with IC\textsubscript{50} values ranging from 0.4 to 6 \textmu M (Tirona et al., 2003). Therefore, the structure feature requirement for being an effective OATPB1 inhibitor is not yet clear. In addition to pravastatin, the likelihood of other statins tested causing clinically significant drug interaction with OATPB1 substrates is low, considering the significantly lower (350- to 11,000-fold) clinically relevant unbound plasma concentration (C\textsubscript{\textup{\textit{a}}}\textsuperscript{max}) compared with its IC\textsubscript{50} for OATPB1 In contrast, inhibition of OATPB1 by cyclosporin A was imputed to the observed clinical interaction between cyclosporin A (an OATP inhibitor) and cerivastatin (an OATPB1 substrate), based on the fact that cyclosporin A had an unbound plasma C\textsubscript{\textup{\textit{a}}}\textsuperscript{max} of 0.1 \textmu M, which approximates to its IC\textsubscript{50} value of 0.2 \textmu M toward OATPB1-mediated transport (Shitara et al., 2003).

In conclusion, the present study showed for the first time that acid versus lactone form of statins, including atorvastatin, lovastatin, and simvastatin, had differential activity toward P-glycoprotein, MR2/Mrp2, and OATP1B1. More specifically, we demonstrated that the lactone form of the statins appeared to be more potent inhibitors of P-glycoprotein and MR2/Mrp2, but less potent inhibitors of OATP1B1 compared with the corresponding acid form. Because both lactone and acid forms of statins are observed in vivo following orally administered statins, the relative composition of acid and lactone forms will potentially influence drug transporter interactions and may ultimately contribute to the differences in pharmacokinetic profiles observed between statins.

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