DIFFERENTIAL INTERACTION OF 3-HYDROXY-3-METHYLGLUTARYL-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) in acid and lactone forms, and pravastatin in acid form only) with multidrug resistance gene 1 (MDR1, ABCB1), p-glycoprotein, multidrug resistance-associated protein 2 (MRP2, ABCC2), and organic anion-transporting polypeptide 1B1 (OATP1B1, SLCO21A6). P-glycoprotein substrate assays were performed using Madin-Darby canine kidney (MDCK) cells expressing MDR1, and the efflux ratios [the ratio of the ratio of basolateral-apical apparent permeability and apical-to-basolateral permeability between MDR1 and MDCK] were 1.87, 2.32/4.46, 2.17/3.17, and 0.93/2.00 for pravastatin, atorvastatin (lactone/acid), lovastatin (lactone/acid), and simvastatin (lactone/acid), respectively, indicating that these compounds are weak or moderate substrates of p-glycoprotein. In the inhibition assays (MDR1, MRP2, MRP2, and OATP1B1), the IC50 values for efflux transporters (MDR1, MRP2, and MRP2) were >100 μM for all statins in acid form except lovastatin acid (>33 μM), and the IC50 values were up to 10-fold lower for the corresponding lactone forms. In contrast, 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 (Corsi 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, and simvastatin are metabolized mainly by CYP3A4 in humans (Corsi 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, acetoxyethyl ester; DMSO, dimethyl sulfoxide A; apical; B, basolateral.
Similar to P-glycoprotein, multidrug resistance-associated protein 2 (MRP2, ABCC2) 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) under alkaline condition. All the other chemicals and reagents were the highest grade available from commercial sources. Structures and physical-chemical properties of statins are summarized in Table 1. Lovastatin and simvastatin was prepared by hydrolysis of the corresponding lactone under alkaline condition. Structures and physical-chemical properties of statins are summarized in Table 1. The acid form of HCl were purchased from Sigma-Aldrich (St. Louis, MO). The acid form of HCl were purchased from Sigma-Aldrich (St. Louis, MO). The acid form of HCl were purchased from Sigma-Aldrich (St. Louis, MO). The acid form of HCl were purchased from Sigma-Aldrich (St. Louis, MO).

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-multwell 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 with 5% CO2 for 24 h before the study. For the grown cells, 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 Victor+ 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)control/(The amount of efflux)untreated × 100, where "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 (% of inhibition)control/(% of inhibition)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/MP2 and MDR1, was used as the positive control.

Inhibition of OATP1B1-Mediated Uptake of Estradiol 17β-β-Glucuronide.

Human embryonic kidney (HEK 293) cells transfected with OATP1B1 were obtained from Professor D. Keppler (University of Heidelberg). Estradiol 17β-β-glucuronide (44 Ci/mmol, >97% purity) was obtained from PerkinElmer Life and Analytical Sciences. On day 1, ~2.5 × 105 HEK 293 cells, transfected with the cDNA for OATP1B1, were seeded on a 24-well poly-β-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-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Molecular Weight</th>
<th>ClogP</th>
<th>pKₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin acid</td>
<td><img src="image1" alt="Structure" /></td>
<td>558.2</td>
<td>4.22</td>
<td>4.30</td>
</tr>
<tr>
<td>Atorvastatin lactone</td>
<td><img src="image2" alt="Structure" /></td>
<td>540.6</td>
<td>4.31</td>
<td>10.5</td>
</tr>
<tr>
<td>Lovastatin acid</td>
<td><img src="image3" alt="Structure" /></td>
<td>422.5</td>
<td>3.71</td>
<td>4.31</td>
</tr>
<tr>
<td>Lovastatin lactone</td>
<td><img src="image4" alt="Structure" /></td>
<td>404.5</td>
<td>4.07</td>
<td>13.5</td>
</tr>
<tr>
<td>Pravastatin</td>
<td><img src="image5" alt="Structure" /></td>
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<td>1.44</td>
<td>4.31</td>
</tr>
<tr>
<td>Simvastatin lactone</td>
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<td>418.6</td>
<td>4.41</td>
<td>13.6</td>
</tr>
<tr>
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<td><img src="image7" alt="Structure" /></td>
<td>436.6</td>
<td>4.05</td>
<td>4.31</td>
</tr>
</tbody>
</table>

*Values were obtained from SciFinder Database.*
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 hydrolysis 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 kinetics 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](https://example.com/fig1.png)

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MDR1-MDCK Efflux Ratio (2 μM)</th>
<th>MDCK B-A/A-B (2 μM)</th>
<th>$K_m$ in ATPase Assay (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pravastatin acid</td>
<td>1.87</td>
<td>1.51</td>
<td>N.A.</td>
</tr>
<tr>
<td>Atorvastatin acid</td>
<td>4.46</td>
<td>2.26</td>
<td>N.A.</td>
</tr>
<tr>
<td>Atorvastatin lactone</td>
<td>2.32</td>
<td>4.31</td>
<td>3.61</td>
</tr>
<tr>
<td>Lovastatin acid</td>
<td>3.17</td>
<td>1.58</td>
<td>N.A.</td>
</tr>
<tr>
<td>Lovastatin lactone</td>
<td>2.17</td>
<td>1.16</td>
<td>11.5</td>
</tr>
<tr>
<td>Simvastatin acid</td>
<td>2.00</td>
<td>1.31</td>
<td>N.A.</td>
</tr>
<tr>
<td>Simvastatin lactone</td>
<td>0.93</td>
<td>0.46</td>
<td>12.3</td>
</tr>
<tr>
<td>Prazosin $^a$</td>
<td>3.50</td>
<td>1.80</td>
<td>not applicable</td>
</tr>
<tr>
<td>Quinidine $^a$</td>
<td>15.0</td>
<td>4.1</td>
<td>not applicable</td>
</tr>
<tr>
<td>Verapamil $^b$</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>7.55</td>
</tr>
</tbody>
</table>

N.A., not available because the compound did not show stimulation of ATP hydrolysis.

$^a$ Positive controls as P-glycoprotein substrates used for the MDR1-MDCK transport assay.

$^b$ Positive control used for the ATPase assay.

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![FIG. 1](https://example.com/fig1.png)

**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.
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.}

![Image](https://i.imgur.com/3.jpg)
Therefore, 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 calcein 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 an 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 atorvastatin acid.

**FIG. 3.** Inhibition of 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.
TABLE 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Unbound C_{max}</th>
<th>IC_{50} MDR1</th>
<th>IC_{50} MRP2</th>
<th>IC_{50} Mrp2</th>
<th>IC_{50} OATP1B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pravastatin acid</td>
<td>0.0648 (^a)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Atorvastatin acid</td>
<td>0.0024 (^a)</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 (^a)</td>
<td>14 ± 1</td>
<td>15 ± 3</td>
<td>15 ± 6</td>
<td>2.6 ± 1.4</td>
</tr>
<tr>
<td>Lovastatin acid</td>
<td>0.0024 (^a)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;33</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>Lovastatin lactone</td>
<td>0.0025 (^a)</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 (^a)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>3.6 ± 1.7</td>
</tr>
<tr>
<td>Simvastatin lactone</td>
<td>0.0041 (^a)</td>
<td>10 ± 8</td>
<td>25 ± 2</td>
<td>17 ± 2</td>
<td>9.7 ± 0.2</td>
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<tr>
<td>Vinblastin(^c)</td>
<td>29</td>
<td>21</td>
<td>21</td>
<td>not applicable</td>
<td>0.23 ± 0.07</td>
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<tr>
<td>Rifampicin SV(^d)</td>
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<td>not applicable</td>
<td>not applicable</td>
<td>0.23 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Based on a 40-mg oral dose. Adapted from data in Corsini et al. (1999).
\(^b\) 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).
\(^c\) Positive control as an inhibitor of MDR1 and MRP/Mrp2 (mean of duplicate experiments).
\(^d\) Positive control as an inhibitor of OATP1B1.
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 (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...
known P-glycoprotein inhibitor is minimal at the intestinal level at which P-glycoprotein expresses. This outcome, using atorvastatin as an example, is because at the clinically relevant doses (20, 40, or 80 mg q.d.), the initial gut concentrations of atorvastatin will be around 300–1150 μM, which is likely to saturate the gut P-glycoprotein-mediated efflux of atorvastatin; the apparent \( K_{m} \) of atorvastatin for P-glycoprotein was 150 μM (Wu et al., 2000). This was supported by the irtraconazole-atorvastatin interaction study (Kantola et al., 1998a) in which an increase of systemic concentrations of atorvastatin and reduced systemic concentrations of its active metabolite, 2-hydroxy atorvastatin, were observed in the presence of irtraconazole compared with the placebo. Since the formation of 2-hydroxy atorvastatin was mediated by CYP3A4 (Lennernas and Fager, 1997), the increased level of parent and the reduced level of the active metabolite in the presence of irtraconazole was most likely due to inhibition of CYP3A4-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 IC50 values for MRP/Mrp2 inhibitors, suggesting that, similar to P-glycoprotein, lipophilicity is likely a favorable physical-chemical feature for MRP/Mrp2 inhibitors. Unfortunately, literature on the structure-requirement as MRP/Mrp2 inhibitors is scarce. Unlike P-glycoprotein, reduced lipophilicity and, possibly, acidic structure and charge will potentially influence drug transporter interactions and may become of major importance in the design of future statin drugs. Administration of statins in their acid form will potentially influence drug transporter interactions and may ultimately contribute to the differences in pharmacokinetic profiles observed between statins.

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