Gut Microbiota-Mediated Drug Interactions between Lovastatin and Antibiotics

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ABSTRACT

Orally administered drugs may be metabolized by intestinal microbial enzymes before absorption into the blood. Accordingly, coadministration of drugs affecting the metabolic activities of gut microbes (e.g., antibiotics) may lead to drug-drug interactions (DDIs). In this study, gut microbiota-mediated DDIs were investigated by studying the pharmacokinetics of lovastatin in antibiotic-treated rats. Incubation of lovastatin with human and rat fecalase preparations produced four metabolites, M1 (demethylbutyryl metabolite), M4 (hydroxylated metabolite), M8 (the active hydroxy acid metabolite), and M9 (hydroxylated M8), indicating involvement of the gut microbiota in lovastatin metabolism. The plasma concentration-time profiles of M8 were compared after oral administration of lovastatin to control rats or those treated with either ampicillin (100 mg/kg) or an antibiotic mixture consisting of cefadroxil (150 mg/kg), oxytetracycline (300 mg/kg), and erythromycin (300 mg/kg). Pharmacokinetic analyses indicated that systemic exposure to M8 was significantly lower in antibiotic-treated rats compared with controls. In addition, fecal M8 formation decreased by 58.3 and 59.9% in the ampicillin- and antibiotic mixture-treated rats, respectively. These results suggested that antibiotic intake may reduce the biotransformation of orally administered drugs by gut microbiota and that the subsequent impact on microbiota metabolism could result in altered systemic concentrations of either the intact drug and/or its metabolite(s).

Introduction

The term "xenobiotic metabolism" refers to the biochemical modification of drugs or food constituents by living organisms, including humans (Croon, 2012). This process generally converts hydrophobic xenobiotics into more hydrophilic products to facilitate their excretion. The liver is known to be a major site of xenobiotic metabolism. However, orally administered xenobiotics may also be metabolized by gut microbial enzymes before being absorbed from the gastrointestinal tract into the blood (Scheline, 1973; Sousa et al., 2008; Haiser and Turbavk, 2013). The role of gut microbiota in the metabolism of orally administered compounds or phytochemicals has been investigated extensively. Representative examples of compounds metabolized by gut microbiota include ginsenosides, cysas, rutin, baicalin, hesperidin, and genistin (Choi et al., 2011; Saad et al., 2012). Some drugs, such as acetaminophen, chloramphenicol, digoxin, and sulfasalazine have also been shown to be affected by gut microbial metabolism (Saad et al., 2012). Once these compounds are orally administered, they are transformed to bioactive, bioinactive, or toxic metabolites by intestinal microbiota before their absorption into the blood (Li and Jia, 2013).

Drug-drug interactions (DDIs) occur when a drug affects the activity of another drug. DDI may result from various processes, including pharmacokinetic and pharmacodynamic interactions. Alteration of drug pharmacokinetics (absorption, distribution, metabolism, and excretion) generally occurs because of inhibition or induction of drug metabolizing enzymes, such as cytochrome P450 enzymes or transporters involved in absorption or excretion processes (Isoherranen et al., 2012; König et al., 2013). However, modulation of gut microbial enzyme activities represents another possible cause of DDI. Thus, drugs (generally antibiotics) affecting gut microbial metabolic activities may alter the pharmacokinetics of coadministered drugs that are metabolized by gut microbiota. As the impact of gut microbiota on drug metabolism has gradually been gaining recognition, the potential DDIs occurring via the interactions with microbiota metabolism studied and the impact of gut microbes on DDIs has been discussed (Lindeman et al., 1981; Saha et al., 1983; Wilson and Nicholson, 2009). Furthermore, the role and significance of microbial β-glucuronidase in connection with enterohepatic recycling of xenobiotics have been emphasized (Robert et al., 2013). The impact of gut microbiota on drug metabolism was evidenced by experimental studies involving animal models, in particular, with germ-free animals and animals in which human microflora had been introduced (Bowey et al., 2003). Lovastatin is a statin, a class of drugs used as cholesterol-lowering agents to reduce cardiovascular disease risk. Lovastatin is a lactone prodrug that is readily hydrolyzed in vivo to yield the active β-hydroxy acid metabolite, a strong inhibitor of 3-hydroxy-3-methylglutaryl

ABBRIVIATIONS: AP, ampicillin; AUC, area under curve; COE, cefadroxil, oxytetracycline, and erythromycin antibiotic mixture; EtOAc, ethylacetate; HPLC, high-performance liquid chromatography; MS/MS, tandem mass spectroscopy; SPE, solid phase extraction.
coenzyme-A reductase (Krukemeyer and Talbert, 1987; Vyas et al., 1990a). In our preliminary study, lovastatin was found to disappear by incubation with human and rat fecalase preparations (unpublished data). Accordingly, we wished to investigate the involvement of gut microbiota in the metabolism of lovastatin, in particular its biotransformation to the β-hydroxy acid metabolite, and the potential for this to associate with antibiotic DDI. In this study, the metabolism of lovastatin by gut microbial enzymes was characterized and the potential for pharmacokinetic DDI between lovastatin and antibiotics was investigated in rats.

Materials and Methods

Lovastatin,Lovastatin β-hydroxyl acid, 4-nitrophenyl-β-D-glucurononoside, and 4-nitrophenyl-β-D-glucopyranoside were purchased from Sigma (St. Louis, MO). Pooled human and rat liver microsomes were obtained from BD Gentest (Woburn, MA).

Subjects. The study subjects were 10 healthy Koreans with an average age of 40.00 ± 9.58 years (range, 23–51 years). These subjects (5 men and 5 women) were nonsmokers who were not taking any medication and who were not regular or current users of antibiotics. The protocols for recruitment of subjects and collection of their stools were approved by the Committee for the Care and Use of Clinical Study in the Medical School, Kyung Hee University (IRB No KHP-2013-03-04-R1).

Animals. Male Sprague-Dawley rats (210–240 g) were supplied by the Koatech Experimental Animal Breeding Center (Gyunggi-do, Korea). All animals were housed in wire cages (two rats per cage) at 20–22°C and 50 ± 10% humidity, fed standard laboratory chow (Samyang Co., Seoul, Korea) and allowed water ad libitum. The rats were segregated into three groups: control group and two antibiotic-treated groups (n = 10 for each). All experiments were performed in accordance with the National Institutes of Health and Kyung Hee University Guidelines for Laboratory Animal Care and Use, and approved by the Committee for the Care and Use of Laboratory Animals in the College of Pharmacy, Kyung Hee University (KHP-2013-03-04-R1).

Fecalase Preparation. Human and rat fecal specimens were prepared for fecalase preparation according to a method previously published by Lee et al. (2002).

Assay of Lovastatin-Metabolizing Activity. The reaction mixture (total volume of 2.5 ml) consisted of 1 ml of 1 mM lovastatin, 1 ml of 0.1 M phosphate buffer, pH 7.0, and 0.5 ml of fecalase suspension. The reaction mixture was incubated at 37°C for 12 hours. The reaction was stopped by the addition of 2.5 ml of MeOH and centrifuged at 3000 g for 10 minutes. The amount ofLovastatin in the resulting supernatant was assayed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a Hewlett Packard series 1050 with an Eclipse Plus C18 column (4.6 × 30 cm) using a UV detector at 254 nm, Agilent Technologies, Santa Clara, CA) and a UV detector at 238 nm (Ramsey, MN). The elution solvent (23% water, 77% acetonitrile) flow rate was 0.6 ml/min for 20 minutes, and the injection volume was 8 μl.

Assay of Fecal β-D-Glucuronidase, β-D-Glucosidase, and α-L-Rhamnosidase Activities. Fecal β-D-glucuronidase, β-D-glucosidase, and α-L-rhamnosidase activities of rat fecalase samples were tested a method previously published by Lee et al. (2002).

Isolation of Lovastatin Metabolites Produced by Intestinal Microbiota. A fresh human stool sample (5 g) was suspended in 45 ml anaerobic dilution broth and centrifuged at 5000 g for 10 minutes. The resulting supernatant was centrifuged at 3000 g for 10 minutes. The pellet was suspended in 1 liter anaerobic dilution broth; lovastatin (0.75 g) was added and aerobically incubated for 5 days. Then the reaction mixture was extracted 3 times (3 × 3 liters) with ethylacetate (EtOAc) and evaporated by a rotary evaporator under vacuum to produce 1.25 g of EtOAc extract. The EtOAc extract was fractionated through a silica gel column (3 × 30 cm) using hexane:EtOAc (1.0 to 1.1); then CHCl3:EtOAc (7.3 to 8.2); and finally CHCl3:MeOH (7.3 to 0.1). Seven fractions (L-1 to L-7) were obtained. The fraction L-3 was loaded onto a silica gel column (1.5 × 20 cm) and eluted using CHCl3:EtOAc (1.0 to 6.4); then CHCl3:acetone (7.3 to 0.1) to produce M8 (2.9 mg). The fraction L-4 was also applied to a silica gel column (1.5 × 20 cm) and eluted using CHCl3:acetone (1.0 to 6.4) and CHCl3:methanol (7.3 to 0.1) to isolate M4 (20.0 mg). The fraction L-6 was loaded onto a silica gel column (1.5 × 20 cm) and eluted using CHCl3:MeOH (1.0 to 0.1) to obtain M1 (2.2 mg). The chemical structures of the isolated compounds were identified by nuclear magnetic resonance (NMR) analysis and comparison of the NMR data (Supplemental Data 1) with published information (Vyas et al., 1990a; Bacher et al., 2009).

Fecal Sample Preparation. Two hundred microliters of rat fecalase samples was percolated through a solid phase extraction (SPE) cartridge (Oasis hydrophilic–lipophilic–balanced 96-well plate; Waters, Milford, MA). The SPE method involved the following steps. First, the SPE cartridge was pre-conditioned with methanol (1 ml) and equilibrated with 0.1% acetic acid (1 ml). The fecalase sample was loaded to the cartridge, which was washed with 0.1% acetic acid (2 × 1 ml). The sample was eluted with 1 ml methanol, and the eluent was evaporated to dryness under an inert stream at 40°C. The residue was dissolved in 100 μl of 0.1% formic acid in acetonitrile and 0.1% formic acid in distilled water (30:70, v/v), and a 5-μl aliquot was injected into the HPLC column for liquid chromatography quadrupole time-of-flight mass spectrometry analyses.

Liver Microsomal Incubation and Sample Preparation. Lovastatin (20 μM, final concentration) was incubated with human or rat liver microsomes (1 mg/ml) in 0.1 M potassium phosphate buffer, pH 7.4, at 37°C for 1 hour in the presence of an NADPH-generating system (0.8 mM NADPH, 10 mM glucose 6-phosphate, and 1 unit of glucose 6-phosphate dehydrogenase). The reaction was stopped by the addition of acetonitrile with 0.1% acetic acid. The reaction solutions were then prepared using SPE, as described above.

Animal Experiments. The control rats were administered water and the antibiotic-treated groups were administered ampicillin (AP; 100 mg/kg) or an antibiotic mixture (COE) consisting of cefadroxil (150 mg/kg), oxytetracycline (300 mg/kg), and erythromycin (300 mg/kg) 3 days before oral administration of either lovastatin (20 mg/kg) or the vehicle. Blood was drawn (0.2 ml) from the tail vein at 0.17, 0.5, 1, 2, 4, 6, 12, and 24 hours after lovastatin or vehicle administration. All samples were stored at −20°C until analysis.

Blood Sample Preparation and Calibration Curves. Two hundred microliters of rat blood samples was centrifuged for 5 minutes at 13,000 g to obtain plasma supernatants. The plasma samples (100 μl) were deproteinized with acetonitrile (200 μl) containing 50 ng/ml digoxin (internal standard). The sample was vigorously vortex-mixed, and then centrifuged at 13,000 g for 5 minutes. The resulting supernatant was transferred to liquid chromatography vials, and a 5-μl aliquot was injected into the liquid chromatography-tandem mass spectrometry analysis system.

Calibration standards were prepared by spiking 10 μl of working standard solutions (lovastatin β-hydroxyl acid) into 90 μl of blank rat plasma at final concentrations of 1–250 ng/ml. The calibration standards were treated as described above before analysis. Calibration curves were generated by plotting the peak area ratio of the analyte to internal standard versus the concentration of the analyte using least-square linear regression. Each standard was prepared in triplicate. The correlation coefficients of the calibration curves were greater than 0.99. The calibration curve equation was y = 0.0390x + 0.0669.

Liquid Chromatography-Tandem Mass Spectrometry Analyses. The liquid chromatography-tandem mass spectrometry conditions used for metabolite identification and pharmacokinetic study are provided as a supplemental material (Supplemental Data 2).

Pharmacokinetic Analysis. The maximum plasma concentration (Cmax) and the time taken to reach Cmax (Tmax) for lovastatin andLovastatin β-hydroxyl acid were estimated directly from the plasma concentration-time profiles. A noncompartmental model provided by WinNonlin Professional 3.1 software (Pharsight Corporation, Mountain View, CA) was used to calculate the area under the plasma drug concentration-time curve (AUC).

Statistics. All the data were expressed as the mean ± standard deviation, and statistical significance was analyzed by one-way analysis of variance followed by Student’s t test.

Results and Discussion

Lovastatin was incubated with human and rat fecalase, and the remaining amount oflovastatin was measured by HPLC analysis. The levels oflovastatin were reduced by 8–19% in the human and rat fecalase samples after a 12-hour incubation. On the basis of these data, the lovastatin-metabolizing activities of human and rat fecalase were...
calculated to be 0.083 ± 0.020 and 0.053 ± 0.017 nmol/h/mg, respectively (Fig. 1).

To investigate the effects of antibiotics on this metabolic activity, antibiotics were orally administered to rats before analysis of fecalase activity. The gut microflora consists of microorganisms that mainly belong to Enterobacteriaceae and the genera Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus, and Bifidobacterium; thus, the microflora has both Gram-positive and Gram-negative organisms (Guarner and Malagelada, 2003). Therefore, broad-spectrum antibiotics that can effectively suppress all types of gut bacteria were chosen. Subsequently, we examined the effects of several selected antibiotics and their combinations on the gut microbiota on the basis of their glycosidase activities and optimized the antibiotic systems (AP and COE) while considering antibiotic-induced adverse physiologic alterations (e.g., diarrhea) and possible pharmacokinetic interference via cytochrome P450 enzymes or drug efflux pumps. AP is a broad-spectrum antibacterial agent that is effective against Gram-positive organisms and some Gram-negative organisms (Wilkowske, 1991). In the case of COE, cefadroxil and oxytetracycline are effective against both Gram-positive and Gram-negative organisms and erythromycin has activities predominantly against Gram-positive bacteria (Wilkowske, 1991). Our data showed that oral administration of AP and COE significantly reduced the lovastatin-metabolizing enzyme activity by 58.3 and 59.9%, respectively, compared with that of control rats. Antibiotic treatment also significantly suppressed other metabolic enzyme activities such as α-D-glucosidase, β-D-glucosidase, and α-L-rhamnosidase activities (Fig. 2).

To investigate the biotransformation of lovastatin by the intestinal microbiota, lovastatin was incubated with human and rat fecalase before analysis using liquid chromatography quadrupole time-of-flight mass spectrometry. Representative data from the human and rat fecalase samples are shown in Fig. 3. The accurate mass data for lovastatin and its postulated metabolites were tabulated in Table 1, and the relevant tandem mass spectrometry (MS/MS) spectra were provided as supplementary data (Supplemental Fig. 2). Four metabolites were detected in the human fecalase samples (Fig. 4), that is, M1, M4, M8, and M9 at m/z 343, m/z 443, m/z 445, and m/z 461, respectively, were detected as protonated ([M+H]+) or sodium adduct ions ([M+Na]+). M1 was postulated as a demethylbutyryl metabolite, based on its accurate mass data (m/z 321.2048, C19H28O4H+); M4 was postulated as an hydroxylated metabolite, based on a molecular weight increase of 16 Da and its MS/MS fragmentation pattern; M8 was identified as an hydroxy acid metabolite, by comparison with the accurate mass data of the authentic reference compound and its MS/MS fragmentation pattern; M9 was postulated as an hydroxylated hydroxy acid metabolite, based on a molecular weight increase of 16 Da compared with M8. The rat fecalase samples also exhibited these 4 metabolites with comparable MS/MS data to those observed in the human samples. M1, M4, and M8 were isolated from the fecalase samples, and their chemical structures were confirmed by 1H and 13C-NMR analyses (Supplemental Data 1). To investigate the actual contribution of microbiota-mediated metabolism to lovastatin pharmacokinetics, the intestinal microbiota-mediated metabolism pattern was compared with those in liver microsomal samples and rat plasma. In
human and rat liver microsomes, 8–9 metabolites were detected (Supplemental Fig. 1, A and B); the human and rat metabolism profiles were comparable, although a slight difference was observed. The chemical structures of the metabolites were determined and/or postulated on the basis of their accurate mass data and MS/MS fragmentation patterns referring to the data available in the literature (Vyas et al., 1990b). The M4 and M8 metabolites identified in human or rat fecal samples were also observed in liver microsomal samples. In rat plasma, a total of 4 metabolites was detected: M1, M6, M8, and M11 (Supplemental Fig. 1C). M1, M8, and M11 were the observed main circulating metabolites, and our findings indicated that gut microbiota-mediated metabolism could have contributed to their plasma level. The accurate mass data for lovastatin and its postulated metabolites were tabulated in Table 1, and their relevant MS/MS spectra were provided as supplemental data (Supplemental Fig. 2).

To investigate the effects of antibiotics on lovastatin pharmacokinetics, plasmalovastatin levels were analyzed after its oral

![Fig. 3. Representative extracted ion chromatograms for lovastatin and its metabolites in human (A) and rat (B) fecalase samples.](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT</th>
<th>Elemental composition</th>
<th>Theoretical Mass</th>
<th>Measured Mass</th>
<th>Error</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lovastatin</td>
<td>15.4</td>
<td>C_{24}H_{36}O_{5}Na^+</td>
<td>427.2482</td>
<td>427.2440</td>
<td>0.7</td>
<td>HF, RF, HLM, RLM</td>
</tr>
<tr>
<td>M1</td>
<td>13.6</td>
<td>C_{19}H_{28}O_{4}H^+</td>
<td>321.2060</td>
<td>321.2048</td>
<td>0.6</td>
<td>HF, RF, RP</td>
</tr>
<tr>
<td>M2</td>
<td>14.7</td>
<td>C_{24}H_{34}O_{5}Na^+</td>
<td>425.2326</td>
<td>425.2321</td>
<td>1.2</td>
<td>HLM, RLM</td>
</tr>
<tr>
<td>M3</td>
<td>9.0</td>
<td>C_{24}H_{36}O_{5}Na^+</td>
<td>443.2404</td>
<td>443.2427</td>
<td>5.2</td>
<td>HLM, RLM</td>
</tr>
<tr>
<td>M4</td>
<td>9.4</td>
<td>C_{24}H_{36}O_{5}Na^+</td>
<td>443.2404</td>
<td>443.2422</td>
<td>4.1</td>
<td>HF, RF, HLM, RLM</td>
</tr>
<tr>
<td>M5</td>
<td>11.5</td>
<td>C_{24}H_{36}O_{5}Na^+</td>
<td>443.2404</td>
<td>443.2426</td>
<td>5.0</td>
<td>RLM</td>
</tr>
<tr>
<td>M6</td>
<td>13.0</td>
<td>C_{24}H_{36}O_{5}Na^+</td>
<td>443.2404</td>
<td>443.2425</td>
<td>4.7</td>
<td>RP, HLM</td>
</tr>
<tr>
<td>M7</td>
<td>14.1</td>
<td>C_{24}H_{36}O_{5}Na^+</td>
<td>443.2404</td>
<td>443.2424</td>
<td>4.5</td>
<td>HLM</td>
</tr>
<tr>
<td>M8</td>
<td>13.6</td>
<td>C_{24}H_{36}O_{5}Na^+</td>
<td>445.2584</td>
<td>445.2584</td>
<td>4.9</td>
<td>HF, RF, HLM, RLM, RP</td>
</tr>
<tr>
<td>M9</td>
<td>5.4</td>
<td>C_{24}H_{38}O_{7}Na^+</td>
<td>461.2510</td>
<td>461.2498</td>
<td>2.6</td>
<td>HF, RF, HLM, RLM</td>
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<tr>
<td>M10</td>
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<td>461.2535</td>
<td>5.4</td>
<td>HLM</td>
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<tr>
<td>M11</td>
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<td>461.2510</td>
<td>461.2538</td>
<td>6.1</td>
<td>HLM, RLM, RP</td>
</tr>
</tbody>
</table>

HF, human feces; RF, rat feces; HLM, human liver microsomes; RLM, rat liver microsomes; RP, rat plasma.
administration to antibiotic-treated rats (AP or COE). Because oral
lovastatin is readily metabolized to the hydroxy acid (the active
metabolite; \textbf{M8}), the plasma concentrations of \textbf{M8} were determined.
The mean plasma concentration-time profiles of \textbf{M8} after oral
lovastatin administration to control rats and those treated with AP or
COE are shown in Fig. 5. The resultant pharmacokinetic parameters
are summarized in Table 2. As shown in Table 2, the $C_{\text{max}}$ values for
\textbf{M8} in control, AP, and COE groups were 188.1 ± 67.8, 114.7 ±
26.0, and 93.1 ± 78.0 ng/ml, and the AUC values were 907.9 ±
309.7, 589.3 ± 117.6, and 448.4 ± 143.7 ng·h/ml, respectively. Thus,
the $C_{\text{max}}$ and systemic exposure (AUC) of the active lovastatin
metabolite, \textbf{M8}, were significantly decreased ($P < 0.05$ and
$P < 0.01$, respectively) in both groups of antibiotic-treated rats compared with
control rats.

The present study investigated the metabolism of lovastatin by gut
microbiota and the consequent pharmacokinetic interactions between
lovastatin and antibiotics. Incubation of lovastatin with fecalase re-
vealed that gut microbial enzymes may be involved in the metabolism
of this drug and may have contributed to the formation of its active
metabolite (\textbf{M8}). The in vitro metabolism profile with liver micro-
somes indicated a considerable overlap between gut microbial and
liver microsomal enzymes in terms of their contributions to lovastatin
metabolism. Furthermore, the in vivo metabolism data suggested that
a considerable portion of circulating lovastatin metabolites, including
the active \textbf{M8} metabolite, could be generated by metabolic processes
mediated by intestinal microbiota. These findings indicated a strong
probability of DDI between lovastatin and antibiotics. The pharma-
cokinetic results of the present study showed that oral administra-
tion of antibiotics before oral lovastatin reduced plasma \textbf{M8} levels.

To rule out any other possible pharmacokinetic interference by anti-
biotics, such as effects on cytochrome P450 enzymes or drug efflux
pumps, lovastatin was administered 3 days after antibiotic treatment,
when the antibiotics should have been eliminated from the body. Al-
though erythromycin, which was included in COE, is considered as
a time-dependent inactivator of CYP3A, it has been found that a sin-
gle dose of erythromycin does not form a metabolic intermediate com-
plex, i.e., it does not inactivate CYP3A4 (Zhang et al., 2010). Thus,
the potential inhibition of the hepatic metabolism of lovastatin by
erthyromycin would be negligible. Furthermore, AP, which does not
have notable DDI issues, also showed comparable effects in \textbf{M8}
pharmacokinetics in this study. This finding shows that the pharma-
cokinetic alteration after antibiotic treatment may be mainly due to an
antibacterial effect rather than inhibition of hepatic pathways. Thus,
the decreased plasma level of \textbf{M8} in rats treated with antibiotics is
likely to have resulted from decreased biotransformation of lovastatin

\begin{table}
\centering
\caption{Pharmacokinetic parameters of lovastatin hydroxy acid in control and antibiotic-
treated rats (\textit{n} = 10).}
\begin{tabular}{|c|c|c|c|}
\hline
 & Control & Ampicillin & Antibiotic Mixture \\
\hline
$C_{\text{max}}$ (ng/ml) & 188.1 ± 67.8 & 114.7 ± 26.0* & 93.12 ± 78.0* \\
$T_{\text{max}}$ (hour) & 2.2 ± 2.2 & 1.9 ± 2.3 & 1.8 ± 1.3 \\
AUC (ng·h/ml) & 907.9 ± 309.7 & 589.3 ± 117.6** & 448.4 ± 143.7*** \\
\hline
\end{tabular}
\end{table}

*P < 0.05. **P < 0.01.
to its hydroxy acid metabolite by gut microbiota. This suggested mechanism was supported by the finding of reduced metabolic activity in antibiotic-treated rat fecalate. Further experiments with germ-free animals and animals in which human flora has been introduced could aid in understanding the contribution of hepatic and gut microbes to M8 formation.

After oral administration, lovastatin is metabolized to the active open-ring lovastatin acid and further metabolized to numerous active or inactive compounds (Krukenmeyer and Talbert, 1987; Vyas et al., 1990b). The systemic bioavailability of the lovastatin acid is only 5%, and the cholesterol-lowering effects of lovastatin are dependent on the production of this acid metabolite (Neuvonen and Jalava, 1996). As lovastatin is mainly metabolized in the liver by CYP3A4, there are well-established DDI between lovastatin and CYP3A4 inhibitors (Neuvonen, 2010). Itraconazole, erthromycin, and grapefruit juice have been shown to significantly elevate serum levels of lovastatin and its hydroxy acid metabolite, potentially leading to serious side effects, such as rhabdomyolysis. The findings of the current study suggest that there is the additional potential for DDI between lovastatin and antibiotics. Although CYP3A4-mediated DDI result in increased systemic exposure to lovastatin and subsequent side effects, antibiotic-induced gut microbiota-mediated DDI reduce bioavailability of the active metabolite, which may attenuate lovastatin’s therapeutic effects. The present study showed a 35–50% decrease in the M8 AUC in antibiotic-treated rats (Table 2). Lovastatin is generally used as a long-term maintenance therapy. Our human fecalase lovastatin metabolism activity data (Fig. 1A) showed considerable interindividual variability. In cases where patients taking lovastatin are treated with antibiotics for a long time, appropriate maintenance of serum cholesterol levels may not be achieved in some individuals. Thus, for some patients, coadministration of antibiotics might lead to serious outcomes due to a failure to control serum cholesterol levels.

Antibiotics also suppressed a range of glycosidase activities (Fig. 2). The suppression of intestinal microbial enzyme activity lasted for at least 3 days after treatment with antibiotics. This indicated that the effects of antibiotics on xenobiotic metabolism might be more extensive and potent than previously recognized. We previously reported that antibiotic treatment altered the pharmacokinetics of hesperidin, a bioactive citrus flavonoid glycoside (Jin et al., 2010). In addition, there have been several reports demonstrating that antibiotics altered the pharmacokinetics of dietary compounds or phytochemicals (Saad et al., 2012). However, studies of this issue using synthetic drugs are still limited. For synthetic drugs, the liver is the primary focus of drug metabolism. Hepatic metabolism generally includes oxidation and conjugation reactions, producing polar low molecular weight products (Croom, 2012). However, our finding suggested that there could be a considerable overlap between intestinal bacterial and hepatic metabolisms in some drugs. Thus, the gut microbiota could act as an organ with a drug-metabolic potential at least equal to that of the liver, depending on the type of drug involved. For this reason, more attention should be paid to gut microbiota-mediated drug metabolism as another possible determinant of DDI.

In conclusion, this study demonstrated the involvement of gut microbiota in the metabolism of lovastatin to its bioactive metabolite. Furthermore, we found that DDI between lovastatin and antibiotics were likely due to antibiotic-mediated inhibition of intestinal bacteria. To our knowledge, this is the first report of gut microbe-mediated lovastatin metabolism and the consequent pharmacokinetic interactions. These findings indicated that administration of antibiotics to patients taking lovastatin may lead to decreased systemic exposure of the active metabolite, reducing its pharmacological effects. Similar antibiotic-induced pharmacokinetic effects may occur with other drugs metabolized by gut microbial enzymes, and further studies on gut microbiota-mediated metabolism are therefore required with a wider range of drugs.

Authorship Contributions


References


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Supplemental data (DMD #58354)

Gut microbiota-mediated drug interactions between lovastatin and antibiotics

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<Legends for Supplemental data>
Supplemental data 1. NMR data for lovastatin metabolites isolated from human fecalase samples
Supplemental data 2. LC-MS/MS conditions for metabolite identification and pharmacokinetic study
Supplemental Figure 1. Extracted ion chromatograms of lovastatin and its metabolites: (A) Human liver microsomes, (B) Rat liver microsomes, and (C) Rat plasma
Supplemental Figure 2. Product ion spectra of lovastatin and its metabolites
Supplemental data 1. NMR data for lovastatin metabolites isolated from human fecalase samples

M1
$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ (ppm) : 6.00 (1H, d, $J = 12.5$ Hz, H-4), 5.79 (1H, dd, $J = 8.0/12.0$ Hz, H-3), 5.52 (1H, brs, H-6), 4.61 (1H, m, H-13), 4.35 (1H, t, $J = 4.5$, H-15), 3.88 (1H, d, $J = 3.5$, H-9), 2.74 (1H, d, $J = 3.0$ Hz, H-16a), 2.70 (1H, d, $J = 6.5$ Hz, H-16b), 2.44 (1H, m, H-2), 2.25 (1H, m, H-14a), 2.10 (1H, m, H-14b), 1.94 (1H, m, H-10a), 1.66 (1H, m, H-8a), 1.45 (1H, m, 14b), 1.25 (1H, m, H-8b), 1.10 (3H, d, $J = 8.5$Hz, H-18), 1.07 (3H, d, $J = 9.5$Hz, H-19).

$^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$ (ppm) : 176.5 (C-17), 133.3 (C-5), 131.5 (C-3), 129.6 (C-4), 128.3 (C-6), 76.4 (C-13), 67.9 (C-9), 62.5 (C-15), 41.5 (C-10), 38.5 (C-16), 37.3 (C-8), 36.6 (C-14), 32.9 (C-1), 30.6 (C-12), 29.7 (C-2), 27.4 (C-7), 26.2 (C-11), 17.8 (C-18), 13.8 (C-19).

M4
$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ (ppm) : 6.05 (1H, d, $J = 12.5$ Hz, H-4), 5.79 (1H, dd, $J = 8.0/12.0$ Hz, H-3), 5.52 (1H, brs, H-9), 5.40 (1H, d, $J = 3.5$, H-6), 4.63 (1H, m, H-13), 4.33 (1H, t, $J = 4.5$, H-15), 2.72 (1H, d, $J = 3.0$ Hz, H-16a), 2.70 (1H, d, $J = 6.5$ Hz, H-16b), 2.44 (1H, m, H-8a), 2.36 (1H, m, H-11), 2.25 (1H, m, H-10), 1.94 (2H, m, H-12), 1.66 (2H, m, H-19), 1.45 (2H, m, H-21),1.40 (1H, m, H-8b), 1.25 (3H, s, H-23), 1.10 (3H, d, $J = 8.5$Hz, H-20), 1.07 (3H, d, $J = 9.5$Hz, H-24), 0.88 (3H, t, $J = 10.0$ Hz, H-22).

$^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$ (ppm) : 178.0 (C-17), 170.5 (C-18), 133.3 (C-5), 131.5 (C-3), 129.6 (C-4), 128.3 (C-6), 77.8 (C-7), 76.4 (C-13), 67.9 (C-9), 62.5 (C-15), 41.5 (C-10), 38.5 (C-16), 37.3 (C-8), 36.6 (C-14), 32.9 (C-12), 30.6 (C-2), 29.7 (C-19), 37.4 (C-1), 24.2 (C-11), 22.7 (C-20), 16.8 (C-22), 27.6 (C-23), 13.8 (C-24), 12.3 (C-21).

M8
$^1$H-NMR (500 MHz, MeOD) $\delta$ (ppm) : 5.98 (1H, d, $J = 10.0$ Hz, H-4); 5.79 (1H, m, H-3), 5.50 (1H, brs-t, H-9), 5.37 (1H, dd, $J = 3.0/6.0$ Hz, H-6), 4.63 (1H, m, H-13), 4.24 (1H, m, H-15), 2.71 (1H, dd, $J = 4.5/17.5$ Hz, H-10), 2.51 (2H, m, H-16), 2.38 (1H, m, H-19), 2.32 (1H, m, H-2), 1.97 (2H, m, H-8), 1.91 (2H, m, H-14), 1.82 (2H, m, H-7), 1.72 (1H, m, H-1), 1.66 (1H, m, H-11a), 1.54 (1H, m, H-12a), 1.43 (1H, m, H-12b), 1.35 (1H, m, H-11b), 1.28 (3H, br, H-23), 1.11 (3H, d, $J = 8.5$Hz, H-20), 1.07 (3H, d, $J = 9.5$Hz, H-24), 0.86 (3H, t, $J = 10.0$ Hz, H-22).

$^{13}$C-NMR (125MHz, MeOD) $\delta$ (ppm) : 176.8 (C-17), 171.9 (C-18), 132.5 (C-6), 131.7 (C-4), 128.9 (C-3), 128.1 (C-5), 76.6 (C-9), 68.1 (C-13), 61.8 (C-15), 41.4 (C-16), 37.7 (C-10), 37.1 (C-8), 36.5 (C-1), 35.2 (C-14), 32.6 (C-19), 32.2 (C-12), 30.5 (C-2), 27.3 (C-7), 26.5 (C-11), 23.7 (C-20), 21.9 (C-21), 15.1 (C-24), 12.6 (C-23), 10.4 (C-22).
Supplemental data 2. LC-MS/MS conditions for metabolite identification and pharmacokinetic study

**LC-QTOF MS analyses for lovastatin metabolites.** The LC-MS system consisted of an Agilent 1260 Infinity HPLC system with an Agilent 6530 Q-TOF mass spectrometer (Agilent Technologies) equipped with a JetStream™ electrospray ionization (ESI) source. Chromatographic separation was achieved with a Hypersil gold column (150 mm × 2.0 mm i.d, 5 μm, Thermo Fisher Scientific Inc., Waltham, MA, USA) and the oven temperature was maintained at 40°C. The mobile phase consisted of 0.1% formic acid (solvent A) and 90% acetonitrile with 0.1% formic acid (solvent B). A gradient program was used for the HPLC separation, with a flow rate of 0.25 mL/min. The initial composition of the mobile phase was 30% solvent B, which was changed to 80% solvent B over 15 min, maintained for 3 min, and followed by a 7-min re-equilibration to the initial condition. The mass spectrometer was operated in positive ion mode. The drying gas (nitrogen) temperature was 300°C, with a flow rate of 10 L/min, and the nebulizer pressure was 20 psi. Capillary and fragmentor voltages were 3500 V and 85 V, respectively. Both centroid and profile data within mass range m/z of 100-500 were acquired at a rate of 4 spectra/s using a Mass Hunter workstation (Agilent Technologies). For targeted MS/MS analysis, the product ion scan range was m/z 50–500. High-purity nitrogen was introduced into a collision cell as the fragmentation gas.

**LC-MS/MS analyses for pharmacokinetic study.** The LC-MS/MS system consisted of an Agilent 1260 Infinity HPLC system with an Agilent 6460 triple-quadrupole mass spectrometer (Agilent Technologies) equipped with a JetStream™ ESI source. Chromatographic separation was achieved with a Poroshell C18 EC120 column (50 mm × 3.0 mm i.d, 2.7μm, Agilent Technologies) and the oven temperature was maintained at 40°C. The mobile phase consisted of 0.1% formic acid (solvent A) and 90% acetonitrile with 0.1% formic acid (solvent B). A gradient program was used for the HPLC separation, with a flow-
rate of 0.3 mL/min. The initial composition of the mobile phase was 30% solvent B, which was changed to 90% solvent B over 3 min, maintained for 3.5 min, and followed by a 4.5-min re-equilibration to the initial condition for 0.1 min. The drying gas (nitrogen) temperature was 300°C, with a flow rate of 10 L/min. The nebulizer pressure was 20 psi, and the capillary voltage was 3500 V. Fragmentor voltages were set at 135 V for lovastatin β-hydroxy acid and 170 V for digoxin (IS). Multiple-reaction monitoring detection was employed using nitrogen as the collision gas; the precursor-product ion pairs monitored were 421.2→100.8 for lovastatin β-hydroxy acid and 825.2→779.2 for digoxin (IS). The retention times of lovastatin β-hydroxy acid and digoxin (IS) were 4.9 and 3.0 min, respectively.
Supplemental Figure 1. Extracted ion chromatograms of lovastatin and its metabolites: (A) Human liver microsomes, (B) Rat liver microsomes, and (C) Rat plasma.
Supplemental Figure 2. Product ion spectra of lovastatin and its metabolites
Supplemental Figure 2 continued
Supplemental Figure 2 continued