Gut microbiota-mediated drug interactions between lovastatin and antibiotics

Dae-Hyeong Yoo, In Sook Kim, Thi Kim Van Le, Il-Hoon Jung, Hye Hyun Yoo, and Dong-Hyun Kim

Department of Pharmacy, College of Pharmacy, Kyung Hee University, Dongdaemun-gu, Seoul 130-701, Korea: DHY, TKVL, IHJ, DHK

Institute of Pharmaceutical Science and Technology and College of Pharmacy, Hanyang University, Ansan, Gyeonggi-do 426-791, Korea: ISK, HHY
Running title: Gut microbiota-mediated drug interactions with lovastatin

Correspondence:

Prof. Dong-Hyun Kim
Department of Pharmacy, College of Pharmacy, Kyung Hee University, Dongdaemun-gu, Seoul 130-701, Republic of Korea
Phone: +82-2-961-0374; Fax: + 82-2-966-2297; E-mail: dhkim@khu.ac.kr

Prof. Hye Hyun Yoo, Ph.D.
Institute of Pharmaceutical Science and Technology and College of Pharmacy, Hanyang University, Gyeonggi-do 426-791, Republic of Korea
Phone: +82-31-400-5804; Fax: + 82-31-400-4783; E-mail: yoohh@hanyang.ac.kr

Number of text pages: 18
Number of Tables: 2
Number of Figures: 5
Number of References: 21
Number of words in the Abstract: 192
Number of words in the Introduction: 511
Number of words in the Discussion: Results and Discussion: 1760

Abbreviations: ESI, electrospray ionization; LC-MS/MS, liquid chromatography-tandem mass spectroscopy.
Abstract

Orally-administered drugs may be metabolized by intestinal microbial enzymes before absorption into the blood. Accordingly, co-administration of drugs affecting the metabolic activities of gut microbes (e.g. antibiotics) may lead to drug-drug interactions (DDI). In this study, gut microbiota-mediated DDI 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, as 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 (Croom, 2012). This process generally converts hydrophobic xenobiotic compounds into more hydrophilic products, in order 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 Turnbaugh, 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, cycasin, rutin, baicalin, hesperidin, and genistin (Saad et al., 2012; Choi et al., 2011). 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, bio-inactive, or toxic metabolites by intestinal microbiota prior to 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 due to inhibition or induction of drug metabolizing enzymes, such as cytochrome P450 enzymes (CYP) or transporters involved in absorption or excretion processes (König et al., 2013; Isoherranen et al., 2012). However, modulation of gut microbial enzyme activities represents another possible cause of DDI. Thus, drugs (generally antibiotics) affecting gut microbe metabolic activities may alter the pharmacokinetics of co-
administered 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 (Lindenbaum 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 (Roberts 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 which is readily hydrolyzed in vivo to yield the active β-hydroxy acid metabolite, a strong inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase (Krukemyer and Talbert, 1987; Vyas et al., 1990). 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

Materials. Lovastatin, lovastatin β-hydroxyl acid, 4-nitrophenyl-β-D-glucuronide, 4-
nitrophenyl-\(\beta\)-D-xylopyranoside, 4-nitrophenyl-\(\alpha\)-L-rhamnopyranoside, and 4-nitrophenyl-\(\beta\)-D-glucopyranoside were purchased from Sigma (St. Louis, MO, USA). Pooled human and rat liver microsomes were obtained from BD Gentest (Woburn, MA, USA).

**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 male and 5 female) were non-smokers 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 NIH and Kyung Hee University Guides 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 h. The reaction
was stopped by the addition of 2.5 mL of MeOH, and centrifuged at 3000 × g for 10 min. The amount of lovastatin 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 × 100 mm i.d., 5.0 μm, Agilent Technologies, Santa Clara, CA, USA) and a UV detector at 238 nm (Ramsey, MN, USA). The elution solvent (23% water, 77% acetonitrile) flow rate was 0.6 mL/min for 20 min 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 500×g for 10 min. The resulting supernatant was centrifuged at 10000×g for 10 min. The pellet was suspended in 1 L anaerobic dilution broth; lovastatin (0.75 g) was added and anaerobically incubated for 5 days. Then the reaction mixture was extracted 3 times (3 × 3 L) 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 cm × 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 cm × 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 (2.0 mg).
The fraction L-6 was loaded onto a silica gel column (1.5 cm × 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 [13-16].

**Fecal sample preparation.** Two hundred microliters of rat fecalase samples were percolated through a solid phase extraction (SPE) cartridge (Oasis hydrophilic-lipophilic-balanced 96-well plate). The SPE method involved the following steps. First, the SPE cartridge was preconditioned 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 nitrogen 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 LC-QTOF MS 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 h 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 h 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 were centrifuged for 5 min at 13000× g to obtain plasma supernatants. The plasma samples (100 µL) were deproteinized with acetonitrile (200 µL) containing 50 ng/mL digoxin (internal standard, IS). The sample was vigorously vortex-mixed, and then centrifuged at 13000 × g for 5 min. The resulting supernatant was transferred to LC vials and a 5-µL aliquot was injected into the LC-MS/MS 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 prior to analysis. Calibration curves were generated by plotting the peak area ratio of the analyte to IS 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 (LC-MS/MS) analyses.** The LC-MS/MS conditions used for metabolite identification and pharmacokinetic study are provided as a supplementary material (Supplemental data 2).

**Pharmacokinetic analysis.** The maximum plasma concentration (\( C_{\text{max}} \)) and the time taken to reach \( C_{\text{max}} \) (\( T_{\text{max}} \)) for lovastatin and lovastatin β-hydroxyl acid were estimated directly from the plasma concentration-time profiles. A non-compartmental model provided by WinNonlin Professional 3.1 software 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 ANOVA, followed by Student’s t-test.

Results and discussion

Lovastatin was incubated with human and rat fecalase and the remaining amount of lovastatin was measured by HPLC anaysis. The levels of lovastatin were reduced by 8-19% in the human and rat fecalase samples after a 12-h incubation. Based on this data, the lovastatin-metabolizing activities of human and rat fecalase were calculated to be 0.083 ± 0.020 nmol/h/mg 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 prior to 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 physiological alterations (e.g., diarrhea) and possible pharmacokinetic interference via CYP 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 to 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 prior to analysis using LC-QTOF/MS. 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 MS/MS spectra were provided as supplementary data (Supplemental Figure 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, as 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 Figure 1A and 1B); 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 KP et al., 1990). 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 Figure 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 supplementary data (Supplemental Figure 2).

To investigate the effects of antibiotics on lovastatin pharmacokinetics, plasma lovastatin levels were analyzed following its oral administration to antibiotic-treated rats (AP or COE). Because oral lovastatin is readily metabolized to the hydroxy acid (the active metabolite; M8), the plasma concentrations of M8 were determined. The mean plasma concentration-time profiles of 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 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, 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 revealed that gut microbial enzymes may be involved in the metabolism of this drug and may have contributed to the formation of its active metabolite (M8). The in vitro metabolism profile with liver microsomes 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 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 pharmacokinetic results of the present study showed that oral administration of antibiotics prior to oral lovastatin reduced plasma M8 levels. To rule out any other possible pharmacokinetic interference by antibiotics, such as effects on CYP enzymes or drug efflux pumps, lovastatin was administered 3 days after antibiotic treatment, when the antibiotics should have been eliminated from the body. Although erythromycin, which was included in COE, is considered as a time-dependent inactivator of CYP3A, it has been found that a single dose of erythromycin does not form a metabolic intermediate complex, i.e., it does not inactivate CYP3A4 (Zhang et al., 2010). Thus, the potential inhibition of the hepatic metabolism of lovastatin by erythromycin would be negligible. Furthermore, AP, which does not have notable DDI issues, also showed comparable effects in M8 pharmacokinetics in this study. This finding shows that the pharmacokinetic alteration following 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 to its hydroxy acid metabolite by gut microbiota. This suggested mechanism was supported by the finding of reduced metabolic activity in antibiotic-treated rat fecalase. 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 \textbf{M8} formation.

Following oral administration, lovastatin is metabolized to the active open-ring lovastatin acid and further metabolized to numerous active or inactive compounds (Krukemyer and Talbert, 1987; Vyas et al., 1990). 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, erythromycin, 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. Whilst 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 \textbf{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 inter-individual 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, co-administration 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 have 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 high molecular weight metabolites. In contrast, bacterial metabolism primarily involves reductive and hydrolytic reactions, generating non-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 lovastatin 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**

*Participated in research design: Kim DH and Yoo HH*

*Conducted experiments: Yoo DH, Le TKV, Kim IS, Jung IH*

*Contributed new reagents or analytic tools: Kim IS*

*Performed data analysis: Yoo DH and Kim IS*

*Wrote or contributed to the writing of the manuscript: Yoo DH, Kim DH and Yoo HH*
References


Footnotes

This research was supported by a grant from Ministry of Food and Drug Safety in 2013 [12182MFDS652].

Dae-Hyeong Yoo and In Sook Kim contributed equally to this work.
Legends for figures

Figure 1. Lovastatin-metabolizing activities of human and rat fecalase. (A) Human fecalase; (B) Rat fecalase from control (NOR) or antibiotic-treated rats. AP, ampicillin; COE, antibiotic mixture [cefadroxil (150 mg/kg), oxytetracycline (300 mg/kg), and erythromycin (300 mg/kg)].

Figure 2. Effects of antibiotics on rat fecalase glycosidase activities. Antibiotics (arrow) were orally administered to rats and (A) β-D-glucuronidase, (B) α-L-rhamnosidase, and (C) β-D-glucosidase activities were measured. Closed circle, saline only; open triangle, ampicillin (AP, 250 mg/kg); open square, antibiotic mixture (COE) [cefadroxil (150 mg/kg), oxytetracycline (300 mg/kg), and erythromycin (300 mg/kg)].

Figure 3. Representative extracted ion chromatograms for lovastatin and its metabolites in (A) human and (B) rat fecalase samples.

Figure 4. Chemical structures of lovastatin and its major metabolites obtained from human and rat fecal samples.

Figure 5. Plasma concentration of lovastatin hydroxy acid in control and antibiotic-treated rats. Lovastatin (20 mg/kg) was orally administered to rats 3 days after a single dose of ampicillin (AP, 250 mg/kg) or antibiotic mixture (COE) (cefadroxil [150 mg/kg], oxytetracycline [300 mg/kg], and erythromycin [300 mg/kg]) (n = 10 for each).
Table 1. Accurate mass data for lovastatin and its metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT</th>
<th>Elemental composition</th>
<th>Theoretical Mass(Da)</th>
<th>Measured mass(Da)</th>
<th>Error</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lovastatin</td>
<td>15.4</td>
<td>C_{24}H_{36}O_5Na^+</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_4H^+</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_5Na^+</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_6Na^+</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_6Na^+</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_6Na^+</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_6Na^+</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_6Na^+</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_{38}O_6Na^+</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_7Na^+</td>
<td>461.2510</td>
<td>461.2498</td>
<td>2.6</td>
<td>HF, RF, HLM, RLM</td>
</tr>
<tr>
<td>M10</td>
<td>7.3</td>
<td>C_{24}H_{38}O_7Na^+</td>
<td>461.2510</td>
<td>461.2535</td>
<td>5.4</td>
<td>HLM</td>
</tr>
<tr>
<td>M11</td>
<td>8.2</td>
<td>C_{24}H_{38}O_7Na^+</td>
<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
Table 2. Pharmacokinetic parameters ofLovastatin hydroxy acid in control and antibiotic-treated rats (n=10)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ampicillin</th>
<th>Antibiotic mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$(ng/mL)</td>
<td>188.1 ± 67.8</td>
<td>114.7 ± 26.0*</td>
<td>93.12 ± 78.0 *</td>
</tr>
<tr>
<td>$T_{\text{max}}$(hr)</td>
<td>2.2 ± 2.2</td>
<td>1.9 ± 2.3</td>
<td>1.8 ± 1.3</td>
</tr>
<tr>
<td>$\text{AUC}(\text{ng}\cdot\text{hr/mL})$</td>
<td>907.9 ± 309.7</td>
<td>589.3 ± 117.6**</td>
<td>448.4 ± 143.7**</td>
</tr>
</tbody>
</table>

* : $P < 0.05$, ** : $P < 0.01$
Figure 1
**Figure 2**

(A) Activity (μmol/min/mg) over time (Day)

(B) Activity (μmol/min/mg) over time (Day)

(C) Activity (μmol/min/mg) over time (Day)
Figure 3
Figure 4
Figure 5