Esterase Inhibition Attribute of Grapefruit Juice Leading to a New Drug Interaction

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ABSTRACT:

This report describes a newly identified potential of grapefruit juice (GFJ) in mediating pharmacokinetic drug interactions due to its capability to inhibit esterase. The study demonstrates that GFJ inhibits purified porcine esterase activity toward p-nitrophenyl acetate and the prodrugs lovastatin and enalapril. In rat and human hepatic or gut S9 fractions and rat gut lumen, GFJ inhibited the hydrolysis of enalaprila and lovastatin, which are known to be metabolized principally by esterases. Lovastatin was metabolized also by CYP3A. In Caco-2 cells, with minimal CYP3A activity, permeability of these prodrugs was increased in the presence of GFJ. In rats, oral coadministration of GFJ or an esterase inhibitor, bis-(p-nitrophenylphosphate), with the prodrugs led to respective increases in plasma area under the curve by 70% or 57% for enalaprilat and 279% or 141% for lovastatin acid. In addition, portal vein-cannulated rats pretreated with GFJ at −15 and −2 h before lovastatin administration (10 mg/kg p.o.) as a solution, 1) in water and 2) in GFJ, showed, respectively, a 49% increase (CYP3A-inhibited) and a 116% increase (both CYP3A and gut esterase-inhibited) in the portal plasma exposure to the active acid, compared with a non-GFJ pretreatment group. Overall, along with the CYP3A inactivation by GFJ, the decreased esterase activity also played a significant role in increasing the metabolic stability and permeability of esters leading to enhancement of exposure to the active drugs in rats. These new esterase inhibition findings indicate that the potential of drug interaction between ester prodrugs and GFJ should also be considered in the clinic.

Since the first report of the grapefruit juice (GFJ) effect on the oral bioavailability of felodipine (Bailey et al., 1989, 1991), the effect of grapefruit juice ingestion on oral pharmacokinetics has been reported for approximately 40 drugs (Saito et al., 2005), generally related to CYP3A inhibition. These drugs differ in their chemical and pharmacological properties but are, in common, extensively metabolized by CYP3A. The mechanism of action thus was postulated to be competitive and mechanism-based inhibition of CYP3A4/5 (hereafter referred to as CYP3A) in the small intestine by GFJ (Schmiedlin-Ren et al., 1997; He et al., 1998). Although some recent reports point to the inhibitory effects of grapefruit juice on the function of P-glycoprotein (Zhou et al., 2004) and OATP (Dresser et al., 2002), the contribution to the bioavailability of drugs that are substrates of P-glycoprotein and OATP has not been well established. It is interesting to note that the magnitude of GFJ effect varied greatly, and we noted that the magnitude of GFJ effect was not proportional to the extent of CYP3A-mediated intestinal metabolism. As an example, cyclosporine, which is extensively metabolized in human intestine by CYP3A, led to only a weak interaction with GFJ (Ducharme et al., 1995), the interaction unlikely to be of clinical significance. However, lovastatin, which is also metabolized in human intestine by CYP3A and esterase, led to one of the most potent and clinically significant drug-GFJ interactions. The oral bioavailability of lovastatin in humans was increased >15-fold by GFJ (Kantola et al., 1998). Because lovastatin is a CYP3A substrate, this interaction was postulated to be the result of CYP3A inhibition. However, because lovastatin (a lactone) is also known to be hydrolyzed by esterase to a hydroxyacid analog (active drug), and the carboxylesterase-mediated hydrolysis of lovastatin is also a major metabolic pathway (Halpin et al., 1993), we hypothesized that the interaction between GFJ and lovastatin is cumulative of CYP3A and esterase inhibition, the attributes of GFJ. There are a few reports on the interactions between esterase inhibitors and the ester compounds, including naturally occurring products. Extract of strawberry and banana juice inhibited the intestinal esterase-mediated hydrolysis of antiviral ester prodrug bis(POC)-PMPA (Van Gelder et al., 1999) and increased absorption of the ester prodrug tenofovir disoproxil in rat ileum by inhibiting its intestinal metabolism (Van Gelder et al., 2000). Intestinal absorption of tenofovir disoproxil fumarate was enhanced also by a defined esters mixture (Van Gelder et al., 2002). However, there are no reports on GFJ-mediated interactions due to esterase inhibition. In this report we used carboxyl esters lovastatin and enalapril as model compounds to investigate the GFJ-
carboxyl ester interactions. Lovastatin, a prodrug for the treatment of hypercholesterolemia, is metabolized by CYP3A4 to oxidative products and hydrolyzed by esterases, e.g., carboxylesterase, to its active form, lovastatin hydroxyacid (hereafter referred to as lovastatin acid) (Halpin et al., 1993). The hydrolysis occurs in gut, liver, and plasma and is considered its major metabolic pathway (Vree et al., 2003). Lovastatin absorption is approximately 30% in humans as well as in rats (Duggan et al., 1989). The similarity of lovastatin absorption in rats and humans led us to employ the rat as a preclinical animal model to elucidate ester-mediated alterations in systemic exposure in rats upon coadministration with GFJ.

The second compound selected for the current studies was enalapril, a prodrug for the treatment of hypertension. Enalaprilat, the hydroxycacid form of this alkyl ester prodrug, is a potent inhibitor of diacid angiotensin-converting enzyme. The bioavailability of enalaprilat in humans (Hockings et al., 1986) is increased to 50 to 60% when given as enalapril, compared with only 3% when enalaprilat is administered orally. Enalapril is metabolized by esterases, e.g., carboxylesterase (Tocco et al., 1982; Drummer et al., 1990), and in vitro and in vivo results indicate that enalapril is primarily absorbed by a nonsaturable, passive diffusion process and is not a suitable model compound for studying transporter-related interaction in rats (Morrison et al., 1996). Thus, enalapril is also considered to be an ideal candidate for testing inhibition of esterase activity with GFJ in the rat model. Evaluation of the esterase-mediated changes in the permeability and metabolism in in vitro human and rat systems, and in vivo rat exposure to enalapril and lovastatin when coadministered with GFJ are described in this report.

Materials and Methods

Materials. GFJ (Minute Maid frozen concentrates, The Coca-Cola Company) cans were purchased locally (Cambridge, MA); enalapril, p-nitrophenyl acetate (PNPA), p-nitrophenol, phenylmethylsulfonyl fluoride (PMSF), and bis-p-nitrophenylphosphate (BNPP) were purchased from Sigma (St. Louis, MO); and enalaprilat was purchased from Toronto Research Chemicals Inc. (Scottdale, PA). A constant lot of GFJ was used throughout the study. The GFJ used in the study was prepared by 1:3 dilution with water, over a 30 cm length from the end of the stomach) of overnight fasted Sprague-Dawley rats obtained from Hilltop Laboratory Animals, Inc. (Scottsdale, PA) (n = 3) twice with 1 ml of phosphate buffer (pH, 7.4). The pH of the pooled lumen was adjusted to 7.0 in a final volume of 7.0 ml. Lumen was used fresh on the day of collection.

Esterase Inhibition in Human and Rat Intestinal and Liver S9 Fractions. Human and rat intestinal or liver S9 fractions (2.0 mg/ml) were incubated at 37°C with enalapril and lovastatin (5 μM) in 0.1 M potassium phosphate buffer, pH 7.4, in the presence or absence of GFJ (pH 7.4; 0, 20, and 40%) in an incubation volume of 0.1 ml. Esterase incubations were quenched after 10 min and lovastatin incubations after 30 min by the addition of 10 μl of glacial acetic acid. The incubation time used was to produce maximal product under linear kinetics conditions.

The quenched mixtures were spiked with varied amounts of GFJ to keep the amount of GFJ equal in each sample. The incubation mixtures then were extracted with 100 μl of 1% acetic acid in acetonitrile containing carbamazepine as an internal standard. The concentration of enalaprilat and lovastatin acid in the supernatants was determined by LC/MS/MS.

Esterase Inhibition by Rat Intestinal Lumen. Rat lumen was collected by rinsing the small intestine (30 cm length from the end of the stomach) of overnight fasted Sprague-Dawley rats obtained from Hilltop Laboratory Animals, Inc. (Scottsdale, PA) (n = 3) twice with 1 ml of phosphate buffer (pH, 7.4). The pH of the pooled lumen was adjusted to 7.0 in a final volume of 7.0 ml. Lumen was used fresh on the day of collection.
A 50-µl aliquot of rat lumen was incubated with lovastatin and enalapril (5 µM) at 37°C in the presence of 50 µl of phosphate buffer (pH 7.0 or 3.5) or 50 µl of GFJ (40% and 80%) at pH 7.0 or 3.5. After 30-min incubations, the reactions were terminated by adding an equal volume of acetonitrile containing carbutamide as the internal standard. The samples were centrifuged at 3000g for 10 min. Supernatants were dried down under nitrogen and the residues were reconstituted into 0.1% formic acid in 10% acetonitrile and analyzed with LC/MS/MS for concentrations of lovastatin acid and enalaprilat.

Permeability in Caco-2 Cells. Caco-2 cell cultures were prepared as described (Xia et al., 2005), and monolayers with the transepithelial electrical resistance values greater than 250 ohm·cm² were used. The single-directional transport studies were performed at 37°C in air. Before each experiment, the confluent cell monolayer on Transwell inserts were washed and equilibrated for 15 min with transport medium at pH 6.0 (to facilitate PEPT1-mediated transport), adding a solution of lovastatin in the transport medium at pH 7.0, or enalapril in transport medium, containing various amounts of GFJ (0, 6.2, 12.5, and 25%), was added to the apical compartment. The uptake study was carried out for 5 min at room temperature and was stopped by aspirating out the solution. The cells were washed three times with cold transport medium and then were lysed with water. The cell lysates were extracted with acetonitrile containing carbutamide as internal standard and centrifuged at 3000g for 10 min. Supernatants were dried down under nitrogen and the residues were reconstituted into 0.1% formic acid in 10% acetonitrile and analyzed with LC/MS/MS for concentrations of lovastatin acid and enalaprilat.

Uptake in Caco-2 Cells. Before the experiment, the confluent cell monolayers on Transwell inserts were washed and equilibrated for 15 min with transport medium, pH 6.0. Enalapril in transport medium, containing various amounts of GFJ (0, 6.2, 12.5, and 25%), was added to the apical compartment. The uptake study was carried out for 5 min at room temperature and was stopped by aspirating out the solution. The cells were washed three times with cold transport medium and then were lysed with water. The cell lysates were extracted with acetonitrile containing carbutamide as internal standard and centrifuged at 3000g for 10 min. The supernatants were collected and analyzed using LC/MS/MS.

Pharmacokinetics Studies. All experiments with rats were performed in accordance with the Institutional Animal Care and Use Committee guidelines and approved by the Committee on Animal Research, Millennium Pharmaceuticals Inc. Male Sprague-Dawley rats, weighing 280 to 350 g, implanted with a jugular vein cannula only or both jugular and portal vein cannulas were obtained from Hilltop Laboratory Animals, Inc. Animals were fasted overnight and for the duration of the study. Water was provided ad libitum.

For oral application, jugular vein-cannulated rats were dosed by gavage (10 ml/kg) with enalapril or lovastatin (10 mg/kg) in 1 water (pH 3.5, the pH of GFJ), 2) GFJ of three strengths (1:3 diluted, 1:2 diluted, concentrate), 3) neutral water, and 4) BNPP (25 mg/kg p.o.) in neutral water. Venous blood samples (0.3 ml) were collected from jugular vein catheters into heparin tubes containing 3 µl of 200 mM PMSF and 5 µl of acetic acid (6:4 with water) predose and at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose. Samples were centrifuged for 5 min at 15,000 rpm, and plasma was collected and frozen at −80°C until analyzed.

For GFJ pretreatment studies, portal vein-cannulated rats pretreated at −15 and −2 h with water (pH 3.5, the pH of GFJ) (group A), and with GFJ (1:3 diluted, 10 ml/kg; groups B and C) were dosed orally with lovastatin (10

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**Fig. 3.** Inhibition of hydrolysis of lovastatin (5 µM) in rat intestinal and liver S9 fractions (A) and intestinal lumen (B) by GFJ.

**Fig. 4.** Inhibition of hydrolysis of enalapril (5 µM) in rat intestinal and liver S9 fractions (A) and intestinal lumen (B) by GFJ.
mg/kg) as a solution in water (pH 3.5) and in GFJ (1:3 diluted). Portal blood samples were collected from portal vein-cannulated rats predose and at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose. Samples were centrifuged for 5 min at 15,000 rpm, and plasma was collected and frozen at −80°C until analyzed.

For intravenous studies, rats with jugular vein catheters were pretreated orally with water (pH 3.5) or GFJ (1:3 diluted; 10 ml/kg) at −0.5 h before a slow i.v. bolus dose of enalapril at 2 mg/kg as a saline solution (5 ml/kg) or lovastatin at 2 mg/kg in a 10:40:10:40 mixture of ethanol, polyethylene glycol 400, N,N-dimethylacetamide, and saline (5 ml/kg). Venous blood samples were collected predose and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose.

**LC/MS/MS Analysis.** The plasma samples were treated with 3 volumes of acetonitrile, containing carbutamide to precipitate protein. Control plasma, with esterase inactivated by the addition of 1% acetic acid and 2 mM PMSF, was used to construct plasma standard curves. Half of the volume of supernatant was dried down under nitrogen and reconstituted with 150 μl of mobile phase B. The mass spectrometer was operated in the multiple reaction monitoring mode using positive ion electrospray ionization. The percentage ofLovastatin acid and Lovastatin hydrolyzed was reduced to 34.4% or <10%, respectively, of the control in human liver S9 fractions by BNPP (10 μM), a positive control, and 38.4% for Lovastatin.

**Inhibitory Effect of GFJ on Hydrolysis by Human Intestinal and Liver S9 Fractions.** The percentage of Lovastatin hydrolyzed was reduced to 54, 46, and 38% of the control in human liver S9 fractions (18.1 pmol/min/mg), and 55, 52, and 47% of the control in human intestinal S9 fractions (7.4 pmol/min/mg) using 10, 20, and 40% GFJ, respectively (Fig. 2A). The percentage of enalapril hydrolyzed was reduced to 87, 90, and 78% of the control in human liver S9 fractions andLiver S9 Fractions.

**Results**

**Effect of GFJ on Purified Porcine Esterase.** The effect of GFJ on the hydrolysis of PNPA, enalapril, and Lovastatin by purified porcine esterase, as measured by the formation of the hydrolysis product, is shown in Fig. 1. The respective hydrolytic activities with 5% and 10% GFJ were decreased to 65% and 54% for PNPA, and the activities with 20% and 40% GFJ were decreased to 50% and 31% for enalapril and 44% and 26% for Lovastatin relative to the control values (310 and 377 pmol/unit/min for enalapril and Lovastatin, respectively). The respective hydrolytic activities with BNPP (10 μM), a positive control, were decreased to <10% for PNPA, <15% for enalapril, and 38.4% for Lovastatin.

**Fig. 5.** Effect of GFJ on Lovastatin A→B permeability across Caco-2 membrane (A) and ratio of Lovastatin acid and Lovastatin in Caco-2 cells (B) at 1 h.

**Fig. 6.** Effect of GFJ on Enalapril A→B permeability across Caco-2 membrane (A) and ratio of Enalaprilat and Enalapril in Caco-2 cells (B) at 1 h.
to 78, 65, and 63% of the control in rat liver S9 fractions (8.8 pmol/min/mg), and 55, 59, and 43% of the control in rat intestinal S9 fractions (1.3 pmol/min/mg) by 10, 20, and 40% GFJ (Fig. 3A), respectively. In rat lumen, lovastatin hydrolysis was reduced to 65% and 48% of the control by 20% and 40% GFJ at pH 7.0 (28.1 pmol/ml/min) (Fig. 4B), and 59% and 24% by 20% and 40% GFJ at pH 3.5 (23.0 pmol/ml/min) (pH of GFJ). Enalapril hydrolysis was reduced to 80, 68, and 49% of the control by 20% and 40% GFJ at pH 7.0 (1.1 pmol/ml/min). The percentage of lovastatin or enalapril hydrolyzed was reduced to 44.7% or 18.6%, respectively, of the control in rat liver S9 fractions by BNPP (100 μM).

**Effect of GFJ on Apical-to-Basal Permeability in Caco-2 Cells.** The permeability of lovastatin was increased by 40% and 22% with 6.25% and 12.5% GFJ (1:3 diluted), but permeability was decreased by 4% and 50% with 25% and 50% GFJ, respectively (Fig. 5A). In Caco-2 cells at 1 h, the intracellularly trapped lovastatin was decreased to 95, 63, 72, and 58%; and lovastatin acid was significantly decreased to 71, 40, 29, and 20% with 6.25, 12.5, 25, and 50% GFJ, respectively. Overall, the corresponding ratio of lovastatin acid to lovastatin (Fig. 5B) was decreased to 81, 64, 42, and 34% by GFJ. The permeability of enalapril was significantly increased by 30, 52, 133, and 88% with 6.25, 12.5, 25, and 50% GFJ, respectively (Fig. 6A). The corresponding intracellularly trapped enalapril in Caco-2 at 1 h was increased to 139, 156, 162, and 187% of the control; and enalapril acid was significantly increased in rats following oral coadministration of enalapril and lovastatin, respectively (10 mg/kg) to rats. The plasma concentration-time profiles of enalaprilat or lovastatin acid following oral coadministration of enalapril or lovastatin (10 mg/kg) with water or with BNPP (25 mg/kg) are shown in Fig. 7, respectively. Similarly, the corresponding ratio of enalaprilat to enalapril (Fig. 6B) was decreased to 64, 49, 35% and 35% of the control.

**Uptake Rate of Enalapril in Caco-2 Cells.** The uptake rate of enalapril in Caco-2 was 1.71 pmol/min/cm², whereas the values were 1.99, 1.91, and 2.02 pmol/min/cm² in the presence of 6.25, 12.5, and 25% GFJ (1:3 diluted), respectively. The uptake rate of enalapril in Caco-2 in the presence of Gly-Sar (a known PEPT1 substrate; 10 mM) did not change significantly, with the value being 1.99 pmol/min/cm².

**Effect of GFJ on Oral PK of Lovastatin Acid and Enalaprilat in Rats.** The plasma concentration-time profiles of enalaprilat or lovastatin acid following oral coadministration of enalapril or lovastatin (10 mg/kg) with water (pH 3.5) or GFJ (1:3 diluted, 1:2 diluted, and the concentrate) are shown in Fig. 8. The Cmax and AUC of lovastatin acid were significantly increased in rats following oral administration of lovastatin with GFJ (Table 1). The AUC was increased by 279, 157, and 170%; and Cmax was increased by 311, 135, and 157% upon coadministration with 1:3 diluted, 1:2 diluted, and concentrated GFJ, respectively. Similarly, the Cmax and AUC of enalaprilat were increased in rats following oral coadministration of enalapril with GFJ (1:3 diluted, 1:2 diluted, and the concentrate), with the respective

**Effect of GFJ on Apical-to-Basal Permeability in Caco-2 Cells.** The permeability of lovastatin was increased by 40% and 22% with 6.25% and 12.5% GFJ (1:3 diluted), but permeability was decreased by 4% and 50% with 25% and 50% GFJ, respectively (Fig. 5A). In Caco-2 cells at 1 h, the intracellularly trapped lovastatin was decreased to 95, 63, 72, and 58%; and lovastatin acid was significantly decreased to 71, 40, 29, and 20% with 6.25, 12.5, 25, and 50% GFJ, respectively. Overall, the corresponding ratio of lovastatin acid to lovastatin (Fig. 5B) was decreased to 81, 64, 42, and 34% by GFJ. The permeability of enalapril was significantly increased by 30, 52, 133, and 88% with 6.25, 12.5, 25, and 50% GFJ, respectively (Fig. 6A). The corresponding intracellularly trapped enalapril in Caco-2 at 1 h was increased to 139, 156, 162, and 187% of the control; and enalapril acid was significantly increased in rats following oral coadministration of enalapril and lovastatin, respectively (10 mg/kg).

<table>
<thead>
<tr>
<th>Coadministration</th>
<th>Enalapril</th>
<th>Lovastatin Acid</th>
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<tbody>
<tr>
<td></td>
<td>AUC:24 h</td>
<td>T_max</td>
</tr>
<tr>
<td></td>
<td>nM·h/h</td>
<td>h</td>
</tr>
<tr>
<td>Water</td>
<td>5743 ± 303</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>BNPP</td>
<td>9006 ± 1292</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>Water (pH 3.5)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>GFJ, 1:3 diluted (pH 3.5)</td>
<td>9465 ± 1886</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>GFJ, 1:2 diluted (pH 3.5)</td>
<td>9743 ± 1560</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>GFJ, concentrate (pH 3.5)</td>
<td>6643 ± 1949</td>
<td>4.0 ± 0.0</td>
</tr>
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</table>

N.D., not determined.
AUCs increased by 65, 70, and 16% (Table 1), and with $C_{\text{max}}$ increased by 60 and 50% (1:3 diluted and 1:2 diluted GFJ), and decreased by 43% by GFJ concentrate, relative to the control.

**Effect of GFJ Pretreatment on Oral PK of Lovastatin in Portal Vein-Cannulated Rats.** The portal vein plasma concentration-time profiles of lovastatin acid plus lovastatin, and 6'β-hydroxylovastatin and its hydroxyacid (6'β-hydroxylovastatin acid) following oral administration of lovastatin (10 mg/kg) to rats pretreated (−2 h and −15 h) either with water (pH 3.5) or 1:3 diluted GFJ showed a graded effect of GFJ pretreatment (Fig. 9). The 6'β-hydroxylovastatin and 6'β-hydroxylovastatin acid were identified by LC/MS/MS spectral comparison with the published data (Halpin et al., 1993) and quantified in plasma using lovastatin standard curves. The portal plasma AUC of lovastatin acid on pretreatment with water (pH 3.5) (group A) versus GFJ (group B) showed a 49% increase in AUC in the GFJ-pretreated rats compared with those pretreated with water (pH 3.5), and the AUC of the corresponding 6'β-hydroxylovastatin plus 6'β-hydroxylovastatin acid decreased to 12% in GFJ-pretreated rats compared with those pretreated with water (pH 3.5) (Table 2). The AUC of lovastatin acid after coadministration of lovastatin with GFJ in rats pretreated with GFJ (group C) showed a 116% increase compared with pretreatment with water (pH 3.5). The portal plasma AUC of lovastatin acid plus lovastatin (group B) showed a 22% increase in AUC in the GFJ-pretreated rats compared with those pretreated with water (pH 3.5). The AUC of lovastatin acid plus lovastatin after coadministration of lovastatin with GFJ in rats pretreated with GFJ (group C) showed an 82% increase compared with pretreatment with water (pH 3.5).
The aqueous solubility of lovastatin is 2.3 mg/mL, and it is known to be an ester prodrug, being hydrolyzed by esterases. In addition, it is also inhibited by esterase activity in vitro in rats and humans and in vivo in rats. Esterases, including carboxylesterases, are ubiquitous enzymes responsible for the metabolism of xenobiotics and endobiotics (Williams et al., 1985). Although other fruit juices are shown to inhibit esterase activity, the literature lacks reports on esterase inhibition by GFJ. This report provides the proof of concept with the demonstration of inhibitory effect of GFJ on PNPA and the prodrugs lovastatin and enalapril, using purified porcine esterase. Esteraligril and lovastatin, two ester prodrugs, are clinically used to increase the bioavailability of the active drugs esteraligril and lovastatin acid which, when administered as such, are poorly absorbed. Lovastatin is known to be hydrolyzed by esterase to its active acid form and to be oxidized by CYP3A (Halpin et al., 1993) as summarized in Fig. 10. A recent study (Chen et al., 2005) also suggested that lovastatin is a weak substrate of Pgp. Thus, the modes of interaction of GFJ with lovastatin in vivo could be CYP3A inhibition, esterase inhibition, and/or Pgp inhibition. Enalapril, on the other hand, is a substrate only for esterases, including hCE1 (Tocco et al., 1982; Drummer et al., 1990) (Fig. 10). The enzyme’s inhibition could lead to increased stability of esters in the lumen and gut enterocytes, resulting in greater absorption of the esters and hence higher exposure to the active acids, via rapid hydrolysis in plasma, for lovastatin and enalapril.

**Esterase Inhibition in Vitro.** The hydrolysis of these esters (measured by the product formation) by purified porcine esterase was inhibited by GFJ in a concentration-dependent manner (Fig. 1). GFJ significantly inhibited the hydrolysis of lovastatin and enalapril also in rat intestinal lumen, and rat and human intestinal and/or liver S9 fractions, as depicted in Figs. 2 to 4. The Caco-2 system was utilized to check whether the increased stability of prodrugs in the gut by GFJ could lead to enhancement of the permeability. The Caco-2 cells used were determined to contain only minor CYP3A activity (data not shown). Thus, effects due to Pgp could be sorted out for lovastatin and enalapril.

**TABLE 2**

Portal plasma PK parameters of lovastatin acid, lovastatin + lovastatin acid, 6'-β-hydroxylavastatin, and its hydroxyacid (6'-β-hydroxylovastatin acid) following oral coadministration of lovastatin (10 mg/kg) with water (pH 3.5) (groups A and B) or GFJ (group C) to portal vein-cannulated rats pretreated at −15 and −2 h with water (pH 3.5) (groups A and B) or GFJ (pH 3.5) (group C)

Data are mean values ± S.D. (n = 3).  

<table>
<thead>
<tr>
<th>Analytes</th>
<th>AUC0–24 h (nM·h)</th>
<th>T_{max} (h)</th>
<th>C_{max} (nM)</th>
<th>AUC0–24 h (nM·h)</th>
<th>T_{max} (h)</th>
<th>C_{max} (nM)</th>
<th>AUC0–24 h (nM·h)</th>
<th>T_{max} (h)</th>
<th>C_{max} (nM)</th>
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</thead>
<tbody>
<tr>
<td>Lovastatin</td>
<td>1949 ± 414</td>
<td>2.4 ± 1.9</td>
<td>611 ± 122</td>
<td>2028 ± 569</td>
<td>2.5 ± 1.0</td>
<td>792 ± 115</td>
<td>1209 ± 883</td>
<td>2.8 ± 2.0</td>
<td>1139 ± 187</td>
</tr>
<tr>
<td>Lovastatin +</td>
<td>2678 ± 515</td>
<td>2.4 ± 1.9</td>
<td>779 ± 140</td>
<td>3265 ± 592</td>
<td>2.5 ± 1.0</td>
<td>944 ± 196</td>
<td>4880 ± 884</td>
<td>2.8 ± 2.0</td>
<td>1306 ± 149</td>
</tr>
<tr>
<td>6'-β-Hydroxylavastatin</td>
<td>1555 ± 290</td>
<td>4.1 ± 2.6</td>
<td>448 ± 118</td>
<td>186 ± 148</td>
<td>1.8 ± 1.7</td>
<td>94.1 ± 34.2</td>
<td>181 ± 62.3</td>
<td>2.3 ± 1.5</td>
<td>114 ± 63.2</td>
</tr>
<tr>
<td>6'-β-Hydroxylavastatin acid</td>
<td>740 ± 125</td>
<td>3.1 ± 2.4</td>
<td>219 ± 65.6</td>
<td>83.1 ± 22.1</td>
<td>2.4 ± 1.9</td>
<td>51.3 ± 21.2</td>
<td>77.0 ± 4.9</td>
<td>2.3 ± 1.5</td>
<td>41.9 ± 13.4</td>
</tr>
</tbody>
</table>

**TABLE 3**

Effect of oral pretreatment with GFJ on PK parameters of lovastatin acid andlovastatin or enalapril and enalaprilat following i.v. administration of lovastatin (2 mg/kg) or enalapril (2 mg/kg) to rats

Data are mean values ± S.D. (n = 3).  

<table>
<thead>
<tr>
<th>Dose</th>
<th>Analytes</th>
<th>Water (pH 3.5) p.o., −0.5 h</th>
<th>GFJ, 1:3 diluted (pH 3.5) p.o., −0.5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0–24 h (nM·h)</td>
<td>CL_{p} (L/h)</td>
<td>V_{dss} (L/h)</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>131 ± 13.0</td>
<td>37.7 ± 3.95</td>
<td>12.5 ± 2.98</td>
</tr>
<tr>
<td>Lovastatin acid</td>
<td>840 ± 154</td>
<td>5.52 ± 1.02</td>
<td>3.61 ± 1.03</td>
</tr>
<tr>
<td>Enalapril</td>
<td>152 ± 29.0</td>
<td>38.8 ± 8.18</td>
<td>3.95 ± 0.76</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>2730 ± 486</td>
<td>2.00 ± 0.31</td>
<td>3.15 ± 1.14</td>
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</tbody>
</table>

It is well known that GFJ inhibits the intestinal CYP3A4 (Schmiedelin-Ren et al., 1997; He et al., 1998), and also Pgp, MRP2, and OATP (Dresser et al., 2002; Honda et al., 2004). The literature is replete with examples of drug interactions with CYP3A (Saito et al., 2005). For the first time, to our knowledge, we demonstrate in this study that GFJ also inhibits esterase activity in vitro in rats and humans and in vivo in rats. Esterases, including carboxylesterases, are ubiquitous enzymes responsible for the metabolism of xenobiotics and endobiotics (Williams et al., 1985). Although other fruit juices are shown to inhibit esterase activity, the literature lacks reports on esterase inhibition by GFJ. This report provides the proof of concept with the demonstration of inhibitory effect of GFJ on PNPA and the prodrugs lovastatin and enalapril, using purified porcine esterase. Esteraligril and lovastatin, two ester prodrugs, are clinically used to increase the bioavailability of the active drugs esteraligril and lovastatin acid which, when administered as such, are poorly absorbed. Lovastatin is known to be hydrolyzed by esterase to its active acid form and to be oxidized by CYP3A (Halpin et al., 1993) as summarized in Fig. 10. A recent study (Chen et al., 2005) also suggested that lovastatin is a weak substrate of Pgp. Thus, the modes of interaction of GFJ with lovastatin in vivo could be CYP3A inhibition, esterase inhibition, and/or Pgp inhibition. Enalapril, on the other hand, is a substrate only for esterases, including hCE1 (Tocco et al., 1982; Drummer et al., 1990) (Fig. 10). The enzyme’s inhibition could lead to increased stability of esters in the lumen and gut enterocytes, resulting in greater absorption of the esters and hence higher exposure to the active acids, via rapid hydrolysis in plasma, for lovastatin and enalapril.
Esterase Inhibition in Vivo. In vivo, the exposure to lovastatin acid was increased by 279, 157, and 170% in rats following oral administration of lovastatin with 1:3 diluted, 1:2 diluted, and concentrated GFJ, respectively, compared with dosing with water (Table 1). At higher concentrations of GFJ, however, a drop in exposure was observed. This is believed to be due to trapping/binding of the drug particles by GFJ pulp, which makes less free drug available for absorption when higher strengths of GFJ are used. This hypothesis was tested by spiking a constant amount of drug in GFJ of various strengths followed by mixing and centrifugation. The concentration of the drug in the centrifugate (pulp-free) was much lower at higher GFJ strengths (88, 74, and 64% of control at 12.5, 25, and 100% GFJ, respectively), supporting our hypothesis. BNPP, a known esterase inhibitor (Walker and Mackness, 1983), produced an effect similar to that of GFJ (Table 1). The exposure to lovastatin acid was increased by 141% in rats following oral coadministration of lovastatin with BNPP compared with coadministration with water. The positive BNPP effect suggested that the esterase plays a significant role in modulating the oral exposure to lovastatin. Thus, the large increase in AUC observed with GFJ was a combination of its effects due to CYP3A and esterase inhibition. To differentiate the modes of GFJ effects via CYP3A inhibition and esterase inhibition, portal vein-cannulated rats were pretreated with GFJ at \( \frac{1}{100} \) and \( \frac{2}{100} \) \( \mu \)g of lovastatin administration to achieve a maximal inactivation of CYP3A activity. 6'β-Hydroxylovastatin, the major CYP3A-mediated metabolite (Vyas et al., 1990), and its hydroxyacid (6'β-hydroxylovastatin acid) (Fig. 10) in portal plasma were determined to qualitatively assess the magnitude of intestinal CYP3A activity involved. The portal exposure of 6'β-hydroxylovastatin and 6'β-hydroxylovastatin acid was significantly decreased to ~11% (Fig. 9, C and D; Table 2), as expected, upon pretreatment with GFJ compared with the pretreatment with water. As a result of inhibition of CYP3A by GFJ pretreatment, the portal plasma exposure toLovastatin acid was increased by 49% (Table 2, groups A and B). When lovastatin was coadministered with GFJ (group C) to portal vein-cannulated rats pretreated with GFJ at ~15 and ~2 h, the portal plasma exposure to CYP3A-mediated products 6'β-hydroxylovastatin and 6'β-hydroxylovastatin acid was generally unchanged, compared with group B rats, as shown in Table 2, indicating that the CYP3A activity remained inhibited by the pretreatment regimen. However, the portal plasma AUC oflovastatin acid in rats coadministered with GFJ (group C) was further increased by 45% (Table 2). The additional 45% exposure increase in group C is postulated to be the result of inhibition of gut lumen and enterocyte esterases by the coadministered GFJ. The quantitation of lovastatin plusLovastatin acid showed a similar trend. Thus, both CYP3A inhibition and esterase inhibition by GFJ led to similar degrees of exposure increases forLovastatin and the active drugLovastatin acid in rats.

Fig. 10. Abbreviated metabolic schemes for enalapril (A) and lovastatin (B) in rat and human liver microsomes.
The exposure to enalaprilat was similarly increased by 65 and 70% in rats following oral coadministration of enalapril with 1:3 and 1:2 diluted GFJ, compared with coadministration with water alone (Fig. 9; Table 1). Enalapril is metabolized by carboxylesterase (hCE1) and is not a CYP3A substrate; thus, the esterase inhibition by GFJ led to the oral exposure increase of enalaprilat in rats. This is consistent with the increased exposure of enalapril when coadministered with the esterase inhibitor BNPP, as well as with the in vitro data. When GFJ was administered orally 0.5 h before the i.v. administration of lovastatin and enalapril to rats, the oral exposure increase of enalaprilat in rats. This is consistent with the increased exposure of enalapril when coadministered with the esterase inhibitor BNPP, as well as with the in vitro data. When GFJ was administered orally 0.5 h before the i.v. administration of lovastatin and enalapril to rats, the i.v. pharmacokinetic parameters remained unaltered, indicating that single oral administration of GFJ did not change the esterase activity at the hepatic level. Thus, the AUC increases after GFJ administration are mainly the results of GFJ effects at the gut lumen and enterocyte level.

Overall, the current study, for the first time (to our knowledge), demonstrated that GFJ decreased lovastatin and enalapril hydrolysis in the gut, and thereby markedly increased the metabolic stability and permeability of the esters, leading to the enhancement of exposure to lovastatin acid and enalaprilat in rats. In the case of lovastatin, the contribution of esterase inhibition was similar to that of CYP3A inhibition. Similarly, the esterase inhibition by GFJ could also lead to oral exposure increases of lovastatin acid and enalaprilat in humans. Moreover, it is proposed that other ester prodrugs are also likely to show drug interactions mediated via the esterase inhibition attribute of GFJ in the clinic.

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References


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