Esterase Inhibition by Grapefruit Juice Flavonoids Leading to a New Drug Interaction

Ping Li, Patrick S. Callery, Liang-Shang Gan, and Suresh K. Balani

Drug Metabolism and Pharmacokinetics, Drug Safety and Disposition, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts (P.L., L.-S.G., S.K.B.); and Department of Basic Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, West Virginia (P.L., P.S.C.)

Received November 14, 2006; accepted April 19, 2007

ABSTRACT:

Our previous studies described a newly identified potential of grapefruit juice (GFJ) in mediating pharmacokinetic drug interactions due to its capability of esterase inhibition. The current study identifies the active components in GFJ responsible for its esterase-inhibitory effect. The esterase-inhibitory potential of 10 constitutive flavonoids and furanocoumarins toward p-nitrophenylacetate (PNPA) hydrolysis was investigated. The furanocoumarins bergamottin, 6',7'-dihydroxybergamottin, and bergapten, and the glycoside flavonoids naringin and hesperidin, at concentrations found in GFJ or higher, did not inhibit the hydrolysis of PNPA by purified porcine esterase and human liver microsomes. However, the flavonoid aglycones morin, galangin, kaempferol, quercetin, 7,8-dihydroxybergamottin, and bergapten, and naringenin showed appreciable inhibition of PNPA hydrolysis in purified porcine esterase, and human and rat liver systems. In Caco-2 cells, demonstrated to contain minimal CYP3A activity, the permeability coefficient of the prodrugs lovastatin and enalapril was increased in the presence of the active flavonoids kaempferol and naringenin, consistent with inhibition of esterase activity. In rats, oral coadministration of kaempferol and naringenin with these prodrugs led to significant increases in plasma exposure to the active acids. In addition, in portal vein-cannulated rats, coadministration of lovastatin with kaempferol (10 mg/kg) led to a 154% and a 113% increase in the portal plasma exposure to the prodrug and active acid, respectively, compared with coadministration with water. The contribution of CYP3A inhibition was demonstrated to be minimal. Overall, a series of flavonoids present in GFJ are identified as esterase inhibitors, of which kaempferol and naringenin are shown to mediate pharmacokinetic drug interaction with the prodrugs lovastatin and enalapril due to their capability of esterase inhibition.

Since the first report of the GFJ effect on the oral bioavailability of felodipine (Bailey et al., 1989, 1991), many studies to identify the active components responsible for the GFJ effects have been reported (Bailey et al., 1993; Edwards et al., 1996; Fukuda et al., 1997; Schmiedlin-Ren et al., 1997; He et al., 1998; Guo et al., 2000; Ho et al., 2001). GFJ composition varies from variety to variety and from lot to lot and also depends on the preparation method (De Castro et al., 2006). In all cases, the majority of the constituents are flavonoids (Ho et al., 2000). Naringin, a predominant constituent in GFJ, is present in concentrations up to 2000 μM (Ross et al., 2000). Even flavonoids of relatively low abundance such as quercetin exist in the 20 μM range. Bergamottin and 6',7'-dihydroxybergamottin (6',7'-DHB), the most abundant furanocoumarin derivatives and another well studied GFJ constituent, are present in concentrations up to 40 μM (Edwards et al., 1996). Although many bioflavonoids inhibited CYP3A in vitro (Ho et al., 2001), in vivo, naringin by itself, at concentrations found in GFJ, was not capable of producing a clinical drug interaction such as that seen with grapefruit juice (Bailey et al., 1998). Several furanocoumarins in grapefruit juice are effective in vitro CYP3A inhibitors and are currently suggested to be clinically active constituents (Schmiedlin-Ren et al., 1997; He et al., 1998).

Our previous study (Li et al., 2007) demonstrated that GFJ inhibits esterase activity and mediates pharmacokinetic interaction with the ester prodrugs lovastatin and enalapril. It is important to identify the active components responsible for this new esterase-mediated GFJ effect in vivo. Several classical esterase inhibitors are known, including diethyl p-nitrophenyl phosphate and bis-p-nitrophenylphosphate (BNPP). However, the extreme toxicity of these compounds precludes their clinical use. Recently, a series of synthesized benzene sulfonamides and the aromatic dione family were identified as selective inhibitors of carboxylesterases (Wadkins et al., 2004, 2005). Likewise, flavoring ester mixtures in strawberry juice were also reported to interact with the prodrug tenofovir, leading to enhanced permeability across Caco-2 (van Gelder et al., 2002). Our recent report (Li et al., 2007) demonstrated that GFJ decreased lovastatin and enalapril hydrolysis in the gut and thereby markedly increased metabolic stability and permeability of esters, leading to the enhancement of exposure to lovastatin acid and enalaprilat in rats. In the current report, the esterase inhibition potential of 10 grapefruit juice components toward

ABBREVIATIONS: GFJ, grapefruit juice; AUC, area under the curve; BNPP, bis-(p-nitrophenyl phosphate); PMSF, phenylmethylsulfonyl fluoride; PNPA, p-nitrophenylacetate; 6',7'-DHB, 6',7'-dihydroxybergamottin; LC/MS/MS, liquid chromatography-tandem mass spectrometry; Pgp, P-glycoprotein; GF120918, N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isooquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide; PK, pharmacokinetic; A-to-B, apical-to-basal.
lovasatin acid in the supernatants were analyzed with LC/MS/MS.

described previously (Xia et al., 2005). Single directional transport studies were initiated by adding 0.1 M potassium phosphate buffer pH 7.4 were incubated at 37°C with PNPA (667 μM) and one of the 10 GJF components of 10 different concentrations: bergamottin (0–100 μM), bergapten (0–100 μM), 6',7'-DHB (0–100 μM), kaempferol (0–50 μM), quercetin (0–50 μM), morin (0–50 μM), galangin (0–50 μM), naringenin (0–200 μM), hesperidin (0–200 μM), and naringin (0–1000 μM). The highest concentration of each component used for esterase inhibition evaluation varied based on their solubility in incubation buffer and their concentration found in GJF. The formation of the product, para-nitrophenol, was monitored spectrophotometrically at 405 nm in 2 min.

Inhibitory Effect on Esterase Activity in Rat Liver Microsomes or S9 Fractions. Rat liver microsomes (2.0 mg/ml) or S9 (2.5 mg/ml) in 0.1 M potassium phosphate buffer pH 7.4 were incubated at 37°C with enalapril (5 μM) (using microsomes) or lovastatin (5 μM) (using S9) in the presence or absence of kaempferol (100 μM), naringenin (100 μM), BNPP (100 μM), or PMSF (1000 μM). The reactions were terminated by adding an equal volume of acetonitrile containing 0.5 μM carbamitane (internal standard) after 10 min of incubation. The samples were kept in a refrigerator (4°C) for 30 min and then centrifuged at 3000g for 10 min. The concentrations of enalaprilat or lovastatin acid in the supernatants were analyzed with LC/MS/MS.

Permeability in Caco-2 Membrane. Caco-2 cell cultures were prepared as described previously (Xia et al., 2005). Single directional transport studies were performed at 37°C in air. Before each experiment, the confluent cell monolayer on Transwell inserts was washed and equilibrated for 30 min with transport medium (Hanks’ balanced salt solution containing 10 mM HEPES and 10 mM glucose pH 7.4). The experiment (n = 4) was initiated by adding a solution of lovastatin (20 μM final concentration) in the transport medium at pH 7.0 or enalapril (20 μM final concentration) in the transport medium at pH 6.0 (lower pH to facilitate PEPT1-mediated transport), containing various amounts of kaempferol or naringenin (50 or 250 μM final concentration), to the apical compartment. To evaluate the Pgp effect on lovastatin permeability, GF120918 (2 μM final concentrations in the incubation) was added into lovastatin stock solution on the donor side and buffer on the receiver side. At 15, 30, 45, and 60 min, aliquots were withdrawn from the basolateral side and replaced immediately with an equal amount of fresh transport medium, except at the 60-min time point (the end of the incubation). After the permeability studies described in the previous section, transendothelial electrical resistance values were measured again to assure the integrity of the cells. The cells were washed three times with cold transport medium and then were lysed with 1% acetic acid in water. The cell lysates were extracted with acetonitrile containing carbamitane as internal standard and centrifuged at 3000g for 10 min. The supernatants were collected and analyzed using LC/MS/MS.

Pharmacokinetics Studies. Pharmacokinetics experiments with enalapril and lovastatin were performed in male Sprague-Dawley rats (Hilltop Laboratory Animals, Inc., Scottdale, PA), weighing 280 to 350 g, that were implanted with either a jugular vein cannula or both jugular and portal vein cannulas. Animals were fasted overnight and for the duration of the study. Water was provided ad libitum. 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.

For systemic exposure studies, jugular vein-cannulated rats (n = 3) were orally dosed by gavage with enalapril and lovastatin (10 mg/kg, 10 ml/kg) in 1) water, 2) kaempferol (2 and 10 mg/kg); and 3) naringenin (2 and 10 mg/kg). 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 diluted with water) predose and at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose. Samples were centrifuged, and plasma samples were collected and frozen at −80°C until analyzed.

For portal exposure studies, portal vein-cannulated rats (n = 4) were orally dosed by gavage with lovastatin (10 mg/kg, 10 ml/kg) in 1) water or 2) kaempferol (10 mg/kg). Portal blood samples (0.3 ml) were collected from portal vein-cannulated rats into heparin tubes containing 3 μl of 200 mM PMSF and 5 μl of acetic acid (6:4 diluted with water) predose and at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h postdose. Samples were centrifuged, and plasma samples were collected and frozen at −80°C until analyzed.

LC/MS/MS Analysis. In vivo plasma samples were protein-precipitated and analyzed with an LC/MS/MS method. Blank plasma, in which esterase was inactivated with 1% acetic acid and 2 mM PMSF, was used to construct plasma standard curves. In general, to 1 volume of plasma was added 3 volumes of acetonitrile containing carbamitane as the internal standard. Samples were vortexed and then centrifuged for 15 min at 3000g. Half of the supernatant was dried down under nitrogen and reconstituted with 150 μl of 5% acetonitrile in 0.1% formic acid. The LC/MS/MS system consisted of a binary high performance liquid chromatography pump (1100; Agilent Technologies, Palo Alto, CA), an HTP PAL autosampler (LEAP Technologies, Carrboro, NC), and a triple-quadrupole mass spectrometer (API-4000; Applied Biosystems, Foster City, CA). Separation was performed on a YMC-ODS-AQ C18 column (30 mm × 2.0 mm; Waters, Milford, MA) using mixtures of formic acid (0.1%) in water and acetonitrile as a mobile phase. The mass spectrometer was operated in the multiple reaction monitoring mode using positive ion electrospray ionization. Multiple reaction monitoring was set at m/z 405.3 → 285.3 for lovastatin, 423.3 → 303.4 for lovastatin acid, 241.4 → 283.4 for 6’β-hydroxyluvastatin, 377.1 → 233.9 for enalapril, and 349.3 → 206.4 for enalaprilot. The quantification limit for enalapril, enalaprilot, lovastatin, and lovastatin acid was generally 2 nM.

Data Analysis. Percentage inhibition of PNPA hydrolysis for each inhibitor was calculated as the ratio of OD at each concentration of inhibitor with respect to that in the absence of the inhibitor, and the percentages were plotted against the concentrations of each tested inhibitor using Prism software (GraphPad Software Inc., San Diego, CA). The sigmoidal dose-response (variable slope) model was used to determine the concentration that gave 50% inhibition (IC50). The IC50 was calculated using the equation Y = minimum activity + (maximum activity − minimum activity)1/(1 + 10[log10 IC50 − X] / Hill slope), where X is the logarithm of concentration and Y is the percentage activity.

PK parameters were calculated by noncompartmental analysis using WinNonlin software version 5.1 (Pharsight, Mountain View, CA). Statistical analysis was performed using Student’s t test. Apparent permeability (Papp cm/s × 10−6) was calculated using the equation Papp = (dQ/dt)/(A × C0), where dQ/dt is total amount transported in the recipient chamber per unit time (e.g., mmol/s), A is surface area (cm2); in our studies A = 0.33 cm2, and C0 is initial donor concentration (e.g., mmol/ml).

Results

Effect of GJF Components on Purified Porcine Esterase and Human Liver Microsomal Hydrolase Activity. Inhibitory activity of GJF components toward esterase activity varied widely. Bergamottin, 6',7'-DHB, and bergapten (each at 100 μM), hesperidin at 200 μM, and naringin at 1000 μM did not show appreciable inhibition of the hydrolysis of PNPA by porcine liver esterase. However, morin, galangin, kaempferol, quercetin, and naringenin showed inhibitory effects. Estimates of IC50 were 1.8 μM for morin, 2.8 μM for galangin, 5.1 μM for kaempferol, 5.9 μM for quercetin, and 110 μM for naringenin (Table 1). Likewise, in human liver microsomes, bergamottin, 6',7'-DHB, bergapten, naringin, and hesperidin did not...
appreciably inhibit the hydrolysis of PNPA. Estimates of \( IC_{50} \) for human liver microsomes were 80 \( \mu \text{M} \) for morin, 81 \( \mu \text{M} \) for galangin, 62 \( \mu \text{M} \) for kaempferol, 43 \( \mu \text{M} \) for quercetin, and 30 \( \mu \text{M} \) for naringenin.

**Effect of Kaempferol and Naringenin on A-to-B Permeability in Caco-2 Cells.** The permeability of lovastatin was not altered by GF120918 alone (0.5%), and was increased by 65, 64, and 66% by kaempferol (250 \( \mu \text{M} \)), naringenin (250 \( \mu \text{M} \)), and the mixture of kaempferol (250 \( \mu \text{M} \)) and GF120918 (2 \( \mu \text{M} \); Fig. 1A). In Caco-2 cells, at 1 h, the intracellularly trapped lovastatin was not significantly altered, with the respective values of 117, 95.8, 105, and 116% of the control (1316 pmol), whereas the trapped amount of lovastatin acid was unaffected by GF120918 alone (120%) and decreased to 54.5, 28.6, and 51.6% by kaempferol, naringenin, and a mixture of kaempferol and GF120918 relative to the control (161 pmol). The overall ratios of lovastatin acid to lovastatin in Caco-2 cells were decreased and are shown in Fig. 1B. The permeability of enalapril was increased by 106, 79.4, 188, and 219% with kaempferol (50 and 250 \( \mu \text{M} \)) and naringenin (50 and 250 \( \mu \text{M} \)), respectively (Fig. 2A). The corresponding intracellularly trapped enalapril in Caco-2 cells at 1 h was increased by 67.3, 69.1, and 26.2%, and decreased by 11% relative to the control (9.9 pmol); and enalaprilat was decreased to 54.5, 36.8, 57.8, and 39.7% relative to the control (41 pmol). The overall ratios of enalaprilat to enalapril were decreased and are shown in Fig. 2B. The amount of lovastatin acid and enalaprilat on the donor side, at the end of the incubation, was very low and was not affected by kaempferol and naringenin.

**Effect of Kaempferol and Naringenin on Enalapril and Lovastatin Hydrolysis in Rat Liver Microsomes or S9 Fractions.** The percentage of lovastatin hydrolyzed in rat liver S9 (8.8 pmol/min/mg) was reduced to 55, 72, 54, and 24% of the control by kaempferol, naringenin, BNPP, and PMSF, respectively (Fig. 3A). Enalapril hydrolysis in rat liver microsomes (62.8 pmol/min/mg) was reduced to 29, 66, 19, and 1% of the control by kaempferol, naringenin, BNPP, and PMSF, respectively (Fig. 3B).

**Effect of Kaempferol and Naringenin on Oral Pharmacokinetics of Lovastatin Acid and Enalaprilat in Rats.** The plasma concentration-time profiles of lovastatin acid and enalaprilat in rats following oral coadministration of lovastatin and enalapril (10 mg/kg) with water, kaempferol (2 and 10 mg/kg), and naringenin (2 and 10 mg/kg) are shown in Figs. 4 and 5. The PK data are shown in Table 2.

**Effect of Kaempferol on the Portal Plasma Pharmacokinetics of Lovastatin.** The portal vein plasma concentration-time profiles of

### TABLE 1

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Porcine Esterase ( IC_{50} ) ( \mu \text{M} )</th>
<th>Human Liver Microsomes ( IC_{50} ) ( \mu \text{M} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaempferol</td>
<td>5.1</td>
<td>62</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5.9</td>
<td>43</td>
</tr>
<tr>
<td>Morin</td>
<td>1.8</td>
<td>80</td>
</tr>
<tr>
<td>Galangin</td>
<td>2.8</td>
<td>81</td>
</tr>
<tr>
<td>Naringenin</td>
<td>110</td>
<td>30</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Naringin</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Bergamottin</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>6,7-DHB</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bergapten</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

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

**Fig. 2.** Effect of kaempferol and naringenin on (A) enalapril A-to-B permeability across Caco-2 membrane, and (B) ratio of enalaprilat and enalapril in Caco-2 cells at 1 h.

**Fig. 3.** Effect of kaempferol and naringenin on enalapril and lovastatin hydrolysis in rat liver microsomes or S9 fractions.
lovastatin, lovastatin acid, and the major oxidative product, 6'-hydroxylovastatin, following oral coadministration of lovastatin (10 mg/kg) to rats with water or kaempferol (10 mg/kg) are shown in Fig. 3. The 6'-hydroxylovastatin was identified by LC/MS/MS spectral comparison with the published data (Halpin et al., 1993) and quantitated in plasma using lovastatin standard curves. The portal plasma PK parameters are shown in Table 3. The AUC ratio of 6'-hydroxylovastatin and lovastatin was 0.064 and 0.078 in rats upon coadministration of lovastatin with water and kaempferol, respectively.

Discussion

6'.β-hydroxylovastatin was identified by LC/MS/MS spectral comparison with the published data (Halpin et al., 1993) and quantitated in plasma using lovastatin standard curves. The portal plasma PK parameters are shown in Table 3. The AUC ratio of 6'.β-hydroxylovastatin and lovastatin was 0.064 and 0.078 in rats upon coadministration of lovastatin with water and kaempferol, respectively.

Inhibition of Esterase Activity in Purified Porcine Esterase and Human Liver Microsomes. The 10 grapefruit components investigated in this study belong to two major and widely known classes, flavonoids (aglycones and glycosides) and furanocoumarins. In the earlier years, grapefruit flavonoids were extensively studied for their CYP3A inhibition potential for understanding the GFJ effect on oral bioavailability of CYP3A substrates. Although these flavonoids inhibited CYP3A4 in vitro, they did not reproduce the grapefruit juice effect when administered orally (Bailey et al., 1993; Rashid et al., 1993). Currently, it is believed that the furanocoumarins, bergamottin and 6',7'-DHB, in GFJ are responsible for the GFJ interaction by competitive and mechanism-based inhibition of CYP3A in the small intestine. These two potent CYP3A inhibitors, in the current study, were found to be devoid of esterase-inhibitory activity in purified porcine esterase and human liver microsomes at 100 μM, a concentration higher than that found in GFJ. Some of the flavonoids, on the other hand, were found to have esterase-inhibitory activity. The effect of flavonoids in aglycone form on carboxylesterases is distinguishable from that of flavonoids in glycoside form. Naringin and hesperidin, the two glycosidic flavonoids, did not inhibit PNPA hydrolysis at
Effect of naringenin (2 and 10 mg/kg) and kaempferol (2 and 10 mg/kg) on PK parameters of enalaprilat and lovastatin acid following oral coadministration with enalapril and lovastatin (10 mg/kg) to rats

Data are mean values ± standard deviation (n = 3).

<table>
<thead>
<tr>
<th>Coadministration</th>
<th>Enalapril</th>
<th>Lovastatin Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{AUC}_{0–8\ h})</td>
<td>(\text{C}_{\text{max}})</td>
</tr>
<tr>
<td>Water</td>
<td>nM \cdot h</td>
<td>h</td>
</tr>
<tr>
<td>Naringenin 2 mg/kg</td>
<td>5494 ± 2309</td>
<td>0.66 ± 0.28</td>
</tr>
<tr>
<td>Naringenin 10 mg/kg</td>
<td>6473 ± 3162</td>
<td>0.83 ± 0.29</td>
</tr>
<tr>
<td>Kaempferol 2 mg/kg</td>
<td>7597 ± 2190</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>Kaempferol 10 mg/kg</td>
<td>11498 ± 3386*</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

* Statistically significant difference (P < 0.05).

Concentrations found in GFJ. However, the five flavonoids morin, galangin, kaempferol, quercetin, and naringenin, aglycone forms, markedly inhibited PNPA hydrolysis by purified porcine esterase, with \(\text{IC}_{50}\) values in the low micromolar range, 1.8 to 110 μM, and by human liver microsomes with \(\text{IC}_{50}\) values in the range of 30 to 81 μM. The higher values with human liver microsomes are believed to be due to a higher protein concentration (lower free concentrations) than that in purified enzyme, and do not necessarily reflect species differences. Nevertheless, these data show that there is a potential of esterase inhibition by these flavonoids in vivo in humans. The combined effects of flavonoids could contribute significantly to the GFJ effects on the pharmacokinetics of ester prodrugs. The effect of kaempferol and naringenin, two of the major and potent esterase-inhibitory flavonoids in GFJ, on the permeability of enalapril and lovastatin in the Caco-2 model, hydrolysis in the rat liver system, and pharmacokinetics in rats were examined. Kaempferol was reported to inhibit CYP3A and Pgp in vitro (Jignesh et al., 2004). Caco-2 cells used in our study were determined to show Pgp and esterase activities, but only a minor CYP3A activity (data not shown). Thus, in this Caco-2 model, the effect of kaempferol on lovastatin could mainly be due to Pgp and/or esterase. Kaempferol and naringenin led to enhancement of the A-to-B permeability of lovastatin and enalapril (Figs. 1A and 2A). GF120918, a Pgp and BCRP inhibitor (Xia et al., 2005), failed to significantly alter lovastatin permeability, suggesting that the contribution of Pgp to the permeability of lovastatin was negligible. Thus, it is mainly the esterase inhibition attribute of kaempferol and naringenin that led to the higher permeability of lovastatin and enalapril. As expected, in these experiments the intracellularly trapped lovastatin acid and enalaprilat were reduced by kaempferol and naringenin, as indicated by decreases in the ratios of lovastatin acid to lovastatin to 59, 27, and 45% by kaempferol, naringenin, and a mixture of kaempferol and GF120918 (Fig. 1B), and by decreases in the ratios of enalaprilat to enalapril to 27, 19, 44, and 38% by kaempferol (50 and 250 μM) and naringenin (50 and 250 μM), respectively (Fig. 2B).

Esterase Inhibition In Vitro and in Vivo in Rats. Hydrolysis of enalapril in rat liver microsomes or hydrolysis of lovastatin in rat liver S9 fraction was inhibited by both kaempferol and naringenin, two of the major flavonoids in GFJ. Kaempferol seemed to be a more potent esterase inhibitor than naringenin in the rat liver system. In vivo, the AUC of lovastatin acid was increased by 171, 246, 159, and 288% in rats following oral administration of lovastatin with kaempferol (2 and 10 mg/kg) and naringenin (2 and 10 mg/kg), respectively, compared with dosing with water (Table 2). Practically no unchanged lovastatin was detected in plasma, because of its rapid hydrolysis by plasma esterases. BNPP, a known esterase inhibitor, produced an effect similar to those of kaempferol and naringenin (Li et al., 2007). 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 kaempferol and naringenin was a combination of their effects due to CYP3A and esterase inhibition. To differentiate the modes of kaempferol and naringenin effects via CYP3A inhibition, esterase inhibition, and/or a combination of both, portal vein-cannulated rats were dosed with water or with kaempferol.
(10 mg/kg). The portal plasma exposure of lovastatin acid, lovastatin, and 6'-β-hydroxylovastatin with kaempferol showed 113, 154, and 208% higher AUC values than did dosing with water. Once absorbed, lovastatin is rapidly converted to lovastatin acid in rat plasma with a half-life shorter than 5 min. Lovastatin is known to be metabolized by CYP3A to 6'-β-hydroxylovastatin as one of the major oxidized products (Halpin et al., 1993). The possible effect of kaempferol on lovastatin exposure by inhibiting intestinal CYP3A was examined by comparing the AUC ratio of oxidized metabolite 6'-β-hydroxylovastatin to lovastatin. The 6'-β-hydroxylovastatin was identified by LC/MS/MS spectral comparison with the published data (Halpin et al., 1993) and quantitated in plasma usingLovastatin standard curves. The AUC ratio of 6'-β-hydroxylovastatin to lovastatin stayed low at 0.064 and 0.078 for coadministration of water and kaempferol, respectively (Table 3), indicating that kaempferol did not markedly inhibit CYP3A activity in rats. This finding is consistent with findings in the literature that kaempferol and naringenin are weak CYP3A inhibitors with IC50 values greater than 100 μM (Ho et al., 2001). In general, the absorption of GFI components is considered to be poor, and their action is postulated to be mainly in the small intestine (Schmiedlin-Ren et al., 1997; He et al., 1998).

The exposure to enalaprilat was also increased by 18, 38, 60, and 109% in rats following oral administration of enalapril with naringenin (2 and 10 mg/kg) and kaempferol (2 and 10 mg/kg), respectively, compared with dosing with water (Fig. 5; Table 2). Enalaprilat is only metabolized by carboxylesterase (hCE1), and is not a CYP3A substrate; thus, the esterase inhibition by kaempferol and naringenin led to an increase of enalaprilat after oral exposure. This is consistent with the increased exposure of enalapril when coadministered with the esterase inhibitor BNPP (Li et al., 2007), as well as with the in vitro data. Overall, the study identified the flavonoids as active components in grapefruit juice that are partly responsible for esterase-inhibitory activity in vitro in rat liver, in human liver and Caco-2 systems, and in vivo in rats. Two of the flavonoids, kaempferol and naringenin, showed significant exposure increases for the active acids in rats when coadministered with lovastatin and enalapril. The results show that the flavonoids have the potential of being used clinically to selectively inhibit esterase activity for enhancing the oral absorption of ester prodrugs.

Acknowledgments. We thank Kym Cardoza for excellent support with animal in-life studies, and Dr. Cindy Q. Xia and Ning Liu for invaluable guidance on transport studies.

References


Address correspondence to: Ping Li, Biogen Idec, Inc. 15 Cambridge Center, Cambridge, MA 02142. E-mail: ping.li@biogenidec.com

TABLE 3

<table>
<thead>
<tr>
<th>Coadministration</th>
<th>Water</th>
<th>Kaempferol,10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0–8 h</td>
<td>Tmax h</td>
</tr>
<tr>
<td></td>
<td>nM · h</td>
<td>nM</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>1440 ± 678</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>Lovastatin acid</td>
<td>3960 ± 482</td>
<td>2.1 ± 2.5</td>
</tr>
<tr>
<td>6'-β-Hydroxylovastatin</td>
<td>93 ± 49</td>
<td>1.6 ± 0.9</td>
</tr>
</tbody>
</table>

* Statistically significantly different (P < 0.05).

Data are mean values ± standard deviation (n = 4).

Downloaded from dbt.aspetjournals.org at ASPET Journals on September 6, 2017.