Tacrolimus, an immunosuppressive agent, is used in patients who undergo transplantation to prevent allograft rejection. Since tacrolimus has a narrow therapeutic range, it is essential to control carefully the blood level of the drug. It has been reported that intake of grapefruit juice (GFJ) leads to an increase in the tacrolimus trough level by 300% (Westveer et al., 1996).

Intestinal absorption of tacrolimus is considered to be limited by a metabolic enzyme, cytochrome P450 3A4, and an efflux transporter, P-glycoprotein (Yokogawa et al., 1999). GFJ is known to inhibit the activities of both intestinal P-glycoprotein and CYP3A4 (Takanaga et al., 1998; Ohnishi et al., 2000). Therefore, the interaction between GFJ and tacrolimus might be attributable to the inhibition of both P-glycoprotein and CYP3A4 in the intestine by GFJ. Pomelo, another citrus fruit of South Asian origin, is ingested popularly in Japan, especially in the eastern part, and other Asian countries, as well as grapefruit.

We have previously reported a case in which intake of pomelo (Cephalocitrus grandis) led to a more than 2-fold increase in the blood level of tacrolimus in a renal transplant recipient (Egashira et al., 2003). Pomelo is botanically close to grapefruit (Cephalocitrus paradisi) and contains furanocoumarins, which have been identified as inhibitors of both CYP3A4 and P-glycoprotein (Guo et al., 2000; Ohnishi et al., 2000). Therefore, it is conceivable that pomelo, as well as grapefruit, inhibits the activities of CYP3A4 and P-glycoprotein. In this study, to elucidate the mechanism of increase in the blood level of tacrolimus by the intake of pomelo, we investigated the effects of pomelo juice extract on the metabolic activity of CYP3A4 and the transport activity of P-glycoprotein.

Materials and Methods

Materials. 6β-Hydroxytestosterone was purchased from Sigma-Aldrich (St. Louis, MO). Testosterone was purchased from Nacalai Tesque Co. (Kyoto, Japan). Human liver microsomes were obtained from BD Gentest (Woburn MA). GFJ and orange juice (OJ) were the products of the Dole Food Company, Inc. (Westlake Village, CA). Pomelo I (C. grandis from Oita; Banpei-yu), pomelo II (C. grandis from Nagasaki; Hirado-Buntan), pomelo III (C. grandis from Kochi; Tosa-Bun- tan) and hassaku (Cephalocitrus hassaku from Oita) were purchased from markets or kindly supplied by farmers. Pomelo I is botanically similar to the pomelo that increased the blood concentration of tacrolimus in our previous report (Egashira et al., 2003). [3H]Digoxin and [14C]inulin were purchased from Amer sham Biosciences UK Ltd. (Little Chalfont, Buckinghamshire, UK). Clarithromycin was kindly supplied by Taishotoyama Pharmaceutical Co., Ltd. (Tokyo, Japan). Tacrolimus, peroxidase-FK506, and anti-FK506 monoclonal antibody were kindly supplied by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). All other chemicals used were commercial products of reagent grade. Bergamottin (BG) and dihydroxybergamottin (DHBG) were extracted from GFJ and purified by the method previously reported (Takanaga et al., 1998).

Extraction of Citrus Fruits with Ethyl Acetate. Pomelo I, II, III, and hassaku were each squeezed by hand to obtain juice. Each juice, or GFJ or OJ, was not pasteurized, and was mixed with 3 volumes of ethyl acetate and shaken vigorously for 10 min. Mixtures were centrifuged at 900g for 10 min, the aqueous phase was discarded, and the organic layer was evaporated to dryness.
under a stream of nitrogen. The residue was reconstituted with methanol to half the initial respective volume of juice. We designated the reconstituted solution as 200% ethyl acetate extracts.

6β-Hydroxylation of Testosterone in Human Liver Microsomes. The activity of CYP3A4 was assayed by measuring the formation of 6β-hydroxytestosterone from testosterone in human liver microsomes according to the method previously reported (Takanaga et al., 2000).

In brief, the reaction was started by addition of human liver microsomes (final concentration 0.1 mg/ml) and testosterone (final concentration 50 µM) to an NADPH-generating system (1 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3 mM MgCl₂) in 100 mM potassium phosphate buffer (pH 7.4) with or without 10% extract of pomelo I, II, III, hassaku, GFJ, or OJ. The mixture was incubated for 15 min at 37°C; then, the reaction was stopped by adding 5 ml of dichloromethane to 400 µl of the mixture, and an aliquot was taken for quantification of 6β-hydroxytestosterone.

To assess the effect of preincubation, the reaction mixture consisting of the NADPH-generating system, human liver microsomes (final concentration 0.1 mg/ml), and 1, 5, or 10% extract of pomelo I, pomelo II, or GFJ in 100 mM potassium phosphate buffer (pH 7.4) was preincubated for 0, 3, 5, or 10 min at 37°C. The reaction was started by 20-fold dilution of this reaction mixture with another mixture containing the NADPH-generating system and testosterone (final concentration 50 µM). The mixture was incubated for 5 min at 37°C; then, the reaction was stopped by the addition of dichloromethane, and an aliquot of the mixture was used for the quantification of 6β-hydroxytestosterone.

Metabolism of Tacrolimus by Human Liver Microsomes. The metabolism of tacrolimus was assayed by measuring the reduction of tacrolimus. In brief, the reaction was started by addition of human liver microsomes (final concentration 0.01 mg/ml) and tacrolimus (final concentration 3 ng/ml) to the NADPH-generating system in 100 mM potassium phosphate buffer (pH 7.4) with or without 1% extract of pomelo I, GFJ, or OJ. The mixture was incubated for 5 min at 37°C; then, 5.58 ml of isomyl alcohol/hexane/methanol (5:250:4, v/v) was added to 400 µl of the reaction mixture to stop the reaction.

To assess the effect of preincubation, the reaction mixture consisting of the NADPH-generating system, human liver microsomes (final concentration 0.01 mg/ml), and 0.1% extract of pomelo I or GFJ in 100 mM potassium phosphate buffer (pH 7.4) was preincubated for 10 min at 37°C. The second reaction was started by 20-fold dilution of this reaction mixture with another mixture containing the NADPH-generating system and tacrolimus (final concentration 3 ng/ml) to the NADPH-generating system in 100 mM potassium phosphate buffer (pH 7.4) with or without 1% extract of pomelo I, GFJ, or OJ. The mixture was incubated for 5 min at 37°C; then, 5.58 ml of isomyl alcohol/hexane/methanol (5:250:4, v/v) was added to 400 µl of the reaction mixture to stop the reaction.

Cell Cultures. The porcine kidney epithelial cell line, LLC-PK1, and LLC-GA5-COL150 cells, which are LLC-PK1 cells transfected with human MDR1-cDNA and overexpressing human P-glycoprotein, were obtained from RIKEN BioResource Center (Ibaraki, Japan). LLC-PK1 and LLC-GA5-COL150 cells were grown in medium 199 (Nikken Bio Medical Laboratory, Kyoto, Japan) containing 10% fetal calf serum, 100 µg/ml streptomycin sulfate, and 270 mg/ml benzylpenicillin K in a humidified atmosphere of 5% CO₂/95% air at 37°C. LLC-GA5-COL150 cells were grown in the presence of 150 ng/ml colchicine.

Transcellular Transport. The transcellular transport experiment was performed as described previously (Wakasugi et al., 1998). LLC-PK1 cells and LLC-GA5-COL150 cells were seeded at 1.0 × 10⁴ cells/cm² on polycarbonate membrane Transwell inserts (1 cm², 3.0-µm pore size; Costar, Cambridge, MA) and cultured for 4 days. They were washed three times with transport buffer (141 mM NaCl, 4.0 mM KCl, 2.8 mM CaCl₂, 1.0 mM MgSO₄, 10 mM glucose, 10 mM HEPES; pH 7.4, 37°C). In the case of the transcellular transport experiment, 1% bovine serum albumin was included in the transport buffer to prevent adsorption of tacrolimus to Transwell, and preincubation was carried out for 15 min in the presence or absence of clathrinomin or the extract of pomelo I or GFJ. The methanol solution of juice extracts was evaporated to dryness. The residue was dissolved in dimethyl sulfoxide and added to the transport buffer to give a final dimethyl sulfoxide concentration of 0.5% and a final juice extract concentration of 50%. In the transport experiment, the transport buffer containing 30 nM [3H]digoxin and 10 µM [14C]ulin or, 1 µM tacrolimus with or without juice extracts or clathrinomin was applied to the donor side, and incubation was performed at 37°C. At the designated time, 0.5 ml of the basolateral or 0.2 ml of the apical side solution was sampled from the receiver side, and an equivalent volume of transport buffer was added as a replacement. To assay the radiolabeled compound, all samples were transferred into counting vials, mixed with scintillation counting fluid (Clear-sol I; Nacalai Tesque, Kyoto, Japan), and placed in a liquid scintillation counter (model LS6500; Beckman Coulter, Fullerton, CA). The concentration of tacrolimus was assayed by enzyme-linked immunosorbent assay.

After the transport study, the cells were washed three times with ice-cold transport buffer. Then, the cells on the filters were solubilized with 0.4 ml of 1 M NaOH, and the solution was neutralized with 0.3 ml of 1 M HCl. The amount of protein in the cells was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard. The apparent transport of [3H]digoxin, [14C]ulin, or tacrolimus was calculated as the permeability (microliters per milligram protein), the ratio of the transported amount per surface area of insert to the initial drug concentration on the donor side. To correct for paracellular transport, the permeability of [14C]ulin was subtracted from the apparent permeability of [3H]digoxin.

Quantification of 6β-Hydroxytestosterone. The sample spiked with organic solvent was shaken vigorously for 5 min and was centrifuged at 740g for 10 min. The organic layer (4 ml) was taken, and the organic solvent was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 µl of mobile phase, and 30 µl of the solution was subjected to HPLC. The HPLC system consisted of a pump (LC-9A; Shimazu, Kyoto, Japan), a spectrophotometer (SPD-10AV; Shimadzu), and a reverse-phase column (Cosmosil, 5C18-MS-II; 4.6 × 150 mm, 5 µm i.d.; Nacalai Tesque, Kyoto, Japan). The mobile phase consisted of 60% methanol/water and was pumped at a flow rate of 1.2 ml/min at 45°C. The wavelength for detection was set at 242 nm.

Quantification of Tacrolimus. The washing buffer consists of phosphate-buffered saline (PBS) and 0.05% Tween 20. Blocking solution (BT-PBS) consisted of the washing buffer and 1% bovine serum albumin. Anti-mouse IgG was diluted with PBS (pH 7.4) to a final concentration of 5 µg/ml (anti-mouse IgG solution). Just before analysis, 30 mg of o-phenylenediamine was dissolved in 30 ml of phosphate-citrate buffer (pH 5.4), and 15 µl of 30% H₂O₂ was added to make the substrate solution. The sample spiked with organic solvent was shaken vigorously for 10 min and centrifuged at 1670g for 5 min. An aliquot (4 ml) of organic solvent was taken, and the organic solvent was evaporated to dryness under a stream of nitrogen. The residue was dissolved in BT-PBS (sample solution). Anti-mouse IgG solution (200 µl) was added to each well of a 96-well plate and left overnight at 2–8°C to fix the antibody. Then, the solution in all wells was washed, the wells were washed five times with washing buffer, and 300 µl of BT-PBS was added to each well and incubated at ambient temperature for 15 min. All the wells were washed three times with washing buffer; then, 100 µl of the sample solution was added to each well of the plate. For the blank plate, 100 µl of BT-PBS was added. An aliquot of 100 µl of peroxidase-FK506 diluted 100-fold with BT-PBS was added to each well. An aliquot of 50 µl of anti-FK506 monoclonal antibody diluted 50-fold with BT-PBS was added to all wells except for the blank plate, to which BT-PBS (50 µl) alone was added. The plates were sealed and left overnight at 2–8°C. Then, the wells were washed five times with washing buffer, and 200 µl of the substrate solution was added to each well. The plate was incubated at ambient temperature in the dark. To stop the reaction, 50 µl of 1.8 M H₂SO₄ was added to each well. Optical density was determined with a plate reader (Microplate reader, model 450; Bio-Rad, Hercules, CA) at the wavelength of 490 nm. Tacrolimus concentrations were calculated from the standard curve fitted to a four-parameter equation: Y = [a−d/(1 + Xⁿ)] + d, where Y is optical density, X is tacrolimus concentration (micrograms per milliliter), and a, b, c, and d are parameters. Tacrolimus standard solution was prepared using an appropriate buffer (100 mM potassium phosphate buffer, pH 7.4, containing human liver microsomes for the metabolism study, or transport buffer containing 1% bovine serum albumin for the transport study) to make final concentrations of 0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 ng/ml.

Quantification of BG and DHBG in Citrus Juices. Pomelo I, II, III, and hassaku were each squeezed by hand. An aliquot of 200 µl of the juice or GFJ was mixed with 4 ml of ethyl acetate and shaken vigorously for 10 min. The mixtures were centrifuged at 900g for 10 min, and 3 ml of the organic layer was taken and evaporated to dryness under a stream of nitrogen. The residue
was dissolved in 300 μl of mobile phase, and 50 μl of the solution was subjected to HPLC. The HPLC system consisted of an LC-9A liquid pump, SPD-10AV spectrophotometer (Shimadzu), and a reversed-phase column (Cosmosil, 5C18-MS-II, 4.6 x 150 mm 5 μm; Nacalai Tesque). For the analysis of BG, the mobile phase was 90% methanol (v/v). For the analysis of DHBG, the mobile phase was 70% methanol (v/v). The mobile phase was pumped at a rate of 1.0 ml/min. Detection was performed by measuring the absorbance at 240 nm (BG) or 254 nm (DHBG).

**Data Analysis.** All data are expressed as means ± S.E.M. Statistical significance was determined with Student's t test or analysis of variance (ANOVA) followed by Dunnett’s test, and a p value of less than 0.05 was considered statistically significant.

**Results**

**Inhibitory Effects of the Ethyl Acetate Extracts of Citrus Juices on the Activity of CYP3A4 and Metabolism of Tacrolimus.** CYP3A4-mediated 6β-hydroxylation of testosterone was significantly decreased in the presence of the 10% ethyl acetate extracts of GFJ, pomelo I, II, III, and hassaku. In contrast, OJ extract did not significantly inhibit 6β-hydroxylation of testosterone (Fig. 1). The extract of GFJ exhibited the most potent inhibition among the juice extracts. Inhibitory effects of pomelo extracts varied with their origin. The inhibitory potency of pomelo I extract was almost equal to that of GFJ extract. Metabolism of tacrolimus was strongly inhibited by 1% ethyl acetate extracts of GFJ and pomelo I (Fig. 2).

**Effect of Preincubation on the Activity of CYP3A4.** Ethyl acetate extracts of pomelo I and II, and GFJ decreased the 6β-hydroxylation of testosterone in both a concentration- and preincubation time-dependent manner (Fig. 3). Preincubation with the ethyl acetate extracts of GFJ and pomelo I for 10 min also potentiated the inhibition of tacrolimus metabolism (Fig. 4).

**Effect of the Ethyl Acetate Extracts of Pomelo I and GFJ and Clarithromycin on the Transcellular Transport of Tacrolimus and [3H]Digoxin across LLC-PK1 and LLC-GA5-COL150 Cell Monolayers.** The basal-to-apical transport of tacrolimus across an LLC-GA5-COL150 cell monolayer was higher than that across an LLC-PK1 cell monolayer, and transport in the opposite direction was lower. The 50% ethyl acetate extract of pomelo I had very little effect on the directional transport of tacrolimus in LLC-GA5-COL150 cells (Fig. 5).

The basal-to-apical transport of [3H]digoxin in LLC-GA5-COL150 cells was higher than that in LLC-PK1 cells and transport in the opposite direction was lower. The basal-to-apical transport of [3H]digoxin was inhibited by the 50% ethyl acetate extracts of pomelo I and GFJ and by clarithromycin, a P-glycoprotein inhibitor, in the LLC-GA5-COL150 cells (Fig. 6). The rank order of the inhibitory potency of these inhibitors was clarithromycin > 50% ethyl acetate extract of GFJ > 50% ethyl acetate extract of pomelo I.

**Concentrations of BG and DHBG in Citrus Fruit Juices.** The contents of BG and DHBG, which are known to be primary CYP3A4 inhibitors in GFJ, pomelo I, II, III, hassaku, and GFJ are shown in Table 1 (Edwards et al., 1996; He et al., 1998). BG was detected in GFJ, pomelo I, and pomelo II at concentrations of 16.4, 12.1, and 0.77 μM, respectively. DHBG was detected in GFJ, pomelo I, and hassaku at concentrations of 16.4, 10.7, and 2.76 μM, respectively. The sum of furanocoumarins, BG and DHBG, contents in six citrus juices significantly correlated with the inhibitory effect of the juices on testosterone 6β-hydroxylation by juice ($r_s = 0.929$, $p = 0.038$, Spearman’s rank analysis).

**Discussion**

Recently, we have reported a case in which the intake of pomelo caused an increase in the blood level of tacrolimus in a renal transplant recipient (Egashira et al., 2003). Since we considered that this increase in the blood level of tacrolimus might be attributable to the inhibition of CYP3A4 and/or P-glycoprotein, we first investigated the inhibitory effect of pomelo on the activity of CYP3A4. All the extracts of pomelo, as well as GFJ extract, significantly inhibited the 6β-hydroxylation of testosterone mediated by CYP3A4. Furthermore, metabolism of tacrolimus itself was almost completely inhibited by the extracts of GFJ and pomelo I. Therefore, a possible explanation for the increase in the blood level of tacrolimus caused by the intake of pomelo is the inhibition of CYP3A4 by pomelo constituents. We investigated the effects of 0.1% to 5% juices on the metabolism of substrates. The concentrations of juice constituents in this study may be much higher than those in plasma. However, the interaction between juices and drugs occurs in the intestinal epithelium, so that the intestinal concentrations of juices are considered to be more relevant to the interaction in vivo. Taking the volume of gastrointestinal tract (3–5 liters) into consideration, the concentrations of juices and their constituents used in this study are considered to be quite feasible and correspond to the intestinal concentration after taking an ordinary amount of juice.

It has been demonstrated that GFJ inhibits the activity of cytochrome P450 through mechanism-based inactivation (Tassaneeyakul...
et al., 2000) and that the inhibitory effect of GFJ lasts more than 3 days in vivo (Takanaga et al., 2000). It is likely that pomelo exerts a similar long-lasting inhibition. Therefore, we investigated the effect of preincubation upon the inhibitory effects of citrus fruit juices on the activity of CYP3A4. Preincubation significantly potentiated the inhibitory effect of the extracts of pomelo I and GFJ on both 6β-hydroxylation of testosterone and metabolism of tacrolimus. These results suggest that the inhibitory effect of pomelo on the metabolism of tacrolimus is exerted in a mechanism-based manner, so that the interaction between pomelo and a substrate of CYP3A4 may be long-lasting (several days). The inhibitory effect of juice extracts on the metabolism of tacrolimus was higher than that on testosterone 6β-hydroxylation. Although the reason for this difference remains to be clarified, it is speculated that juice constituent has a higher affinity to the site of interaction with tacrolimus.

GFJ contains furanocoumarins, BG and DHBG, which inhibit CYP3A4 (Ohnishi et al., 2000). Therefore, we quantified BG and DHBG in GFJ, pomelo I, II, III, and hassaku. The furanocoumarins were detected in all the citrus fruits except for pomelo III, and the contents of BG and DHBG were well correlated with the inhibitory potencies for CYP3A4. GFJ contains both monomer and dimer forms of furanocoumarins. Although the contents of the dimers are smaller than those of the monomers, the dimers are known to be more potent inhibitors of CYP3A4 (Tassaneeyakul et al., 2000). The inhibitory effect of pomelo III, in which BG and DHBG were not detected, may be attributable to dimeric forms of furanocoumarins or other unidentified components. Regrettably, we could not quantitate the contents of dimer forms, because we could not obtain authentic samples.

It is conceivable that the intestinal absorption of tacrolimus is regulated by not only CYP3A4, but also P-glycoprotein. Indeed, Floren et al. (1997) have reported that oral bioavailability of tacrolimus was doubled by the coadministration of ketoconazole, a potent inhibitor of both CYP3A4 and P-glycoprotein, without affecting hepatic bioavailability in humans. In addition, Yokogawa et al. (1999) have reported that the bioavailability of tacrolimus in mdr1a knockout mice was about 3 times higher than that in normal mice. We have also...
FIG. 6. Effects of pomelo I (A and D) and GFJ (B and E) 50% ethyl acetate extracts, and clarithromycin (C and F) on the transcellular transport of digoxin in LLC-PK1 cell (A–C) or LLC-GAS-COL150 cell (D–F) monolayers. The monolayers were incubated for 60 min at 37°C with 23–30 nM [3H]digoxin in the absence (open symbols) or presence (closed symbols) of inhibitors. Inhibitors were added to the medium on both sides of the monolayers during preincubation (15 min) and incubation. [14C]Inulin (10–14 μM) was used to estimate the paracellular flux of [3H]digoxin. Each point represents the mean ± S.E.M (n = 3). *, p < 0.05, significantly different from the control (Student’s t test).

TABLE 1
Contents of furanocoumarins in GFJ and other citrus juices

<table>
<thead>
<tr>
<th>Juice</th>
<th>Concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHBG</td>
</tr>
<tr>
<td>GFJ</td>
<td>16.4</td>
</tr>
<tr>
<td>Pomelo I</td>
<td>10.7</td>
</tr>
<tr>
<td>Pomelo II</td>
<td>N.D.</td>
</tr>
<tr>
<td>Pomelo III</td>
<td>N.D.</td>
</tr>
<tr>
<td>Hassaku</td>
<td>2.76</td>
</tr>
</tbody>
</table>

N.D., not detected.

demonstrated by using rat everted intestine that GFJ inhibits the serosal-to-mucosal efflux of rhodamine 123 and fexofenadine, which are substrates of P-glycoprotein, and do not undergo significant metabolic biotransformation by CYP3A4 (Tian et al., 2002). Since tacrolimus is a substrate of P-glycoprotein, we cannot neglect the contribution of P-glycoprotein to the pharmacokinetics of tacrolimus. However, in LLC-GAS-COL150 cells overexpressing human P-glycoprotein, pomelo I and GFJ extracts had very little effect on the transport of tacrolimus. The basal-to-apical transport of [3H]digoxin was inhibited in the presence of pomelo I and GFJ extracts, although the inhibitory effect of pomelo I extract was weaker than that of GFJ extract. These results are not consistent with the finding of Xu et al. (2003) that the transport of digoxin across Caco-2 cells was inhibited by pomelo as well as grapefruit. They applied directly each juice to the cells, whereas we used the ethyl acetate extracts of juices. Therefore, one of the reasons for this inconsistency is that the inhibition of P-glycoprotein is attributed to a water-soluble component that is not extracted by ethyl acetate. Another reason is that the difference is not extracted by ethyl acetate. An additional reason is the differences in the source, breeding, harvesting conditions, and so on, that may affect the level of constituents. Xu et al. (2003) obtained fruit from local supermarkets in Singapore, so that the aforementioned factors considerably differ from those in Japan. Although digoxin is a well known probe of P-glycoprotein (Johne et al., 1999; Westphal et al., 2000), GFJ had very little effect on the pharmacokinetics of digoxin in humans (Becquemont et al., 2001), whereas clarithromycin, a P-glycoprotein inhibitor, significantly increased the serum level of digoxin (Kurata et al., 2002). These results are in good agreement with our finding that the inhibitory effect of GFJ on P-glycoprotein is weaker than that of clarithromycin. Although clarithromycin is known as a CYP3A4 inhibitor, LLC-PK1 and its derivative cell lines do not express CYP3A4 (Brimer et al., 2000). Overall, the results suggest that pomelo is not likely to increase the blood level of a drug that is a substrate of P-glycoprotein, but not CYP3A4, such as digoxin. The increase in the blood level of tacrolimus by pomelo may be attributable not to the inhibition of P-glycoprotein, but rather to the inhibition of CYP3A4.

In conclusion, pomelo constituents inhibit the activity of CYP3A4 and may lead to an increase in the blood level of orally administered tacrolimus. Therefore, drugs whose kinetics can be altered by grapefruit may also interact with grapefruit-related citrus fruits, such as pomelo.

References


juice and orange juice on the intestinal efflux of P-glycoprotein substrates. *Pharm Res (NY)* **19:**802–809.


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