

# PHARMACOKINETICS AND DRUG DISPOSITION

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## Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine

**Objectives:** Our objective was to examine the effect of different fruits and their constituents on P-glycoprotein and organic anion transporting polypeptide (OATP) activities in vitro and on drug disposition in humans.

**Methods:** P-glycoprotein-mediated digoxin or vinblastine efflux was determined in polarized epithelial cell monolayers. OATP-mediated fexofenadine uptake was measured in a transfected cell line. The oral pharmacokinetics of 120 mg fexofenadine was assessed with water, 25%-strength grapefruit juice, or normal-strength grapefruit, orange, or apple juices (1.2 L over 3 hours) in a randomized 5-way crossover study in 10 healthy subjects.

**Results:** Grapefruit juice and segments and apple juice at 5% of normal strength did not alter P-glycoprotein activity. Grapefruit extract reduced transport. 6',7'-Dihydroxybergamottin had modest inhibitory activity (50% inhibitory concentration [IC<sub>50</sub>], 33 μmol/L). In contrast, grapefruit, orange, and apple juices at 5% of normal strength markedly reduced human OATP and rat oatp activity. 6',7'-Dihydroxybergamottin potently inhibited rat oatp3 and oatp1 (IC<sub>50</sub>, 0.28 μmol/L). Other furanocoumarins and bioflavonoids also reduced rat oatp3 activity. Grapefruit, orange, and apple juices decreased the fexofenadine area under the plasma concentration-time curve (AUC), the peak plasma drug concentration (C<sub>max</sub>), and the urinary excretion values to 30% to 40% of those with water, with no change in the time to reach C<sub>max</sub>, elimination half-life, renal clearance, or urine volume in humans. Change in fexofenadine AUC with juice was variable among individuals and inversely dependent on value with water.

**Conclusions:** Fruit juices and constituents are more potent inhibitors of OATPs than P-glycoprotein activities, which can reduce oral drug bioavailability. Results support a new model of intestinal drug absorption and mechanism of food-drug interaction. (Clin Pharmacol Ther 2002;71:11-20.)

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Clinical response to a drug can vary markedly among and within individuals, ranging from lack of efficacy to toxicity. Because the effect of a drug is largely dependent on its concentration at the tissue site(s) of action, processes that determine drug disposition can have an important therapeutic impact. Metabolizing enzymes can convert a drug into more polar and less active products, resulting in reduced tissue concentration and effect.<sup>1</sup> Efflux transporters such as P-glycoprotein, the product of the human *MDR1* gene, have been shown to limit oral drug absorption and tissue distribution.<sup>2,3</sup> Recent molecular cloning and expression of drug uptake transporters may provide important new insights. The organic anion transporting polypeptides (OATPs) constitute a family of transporters that may play a crucial role.<sup>4</sup> Because there can be overlap of cellular distribution and substrate specificity of drug-metabolizing enzymes and transporters, a complex interplay may determine drug disposition.<sup>5-7</sup>

Since the first report in 1991,<sup>8</sup> it has become well established that the dietary constituent grapefruit juice can produce clinically important interactions with medications by inhibiting cytochrome P4503A4 (CYP3A4)-mediated drug metabolism.<sup>9-11</sup> Recent information suggests that grapefruit juice may also produce drug interactions by modulating the activity of P-glycoprotein. However, the effects are controversial, ranging from activation to inhibition.<sup>12,13</sup> Therefore our current understanding of the effect of diet on drug transporters is incomplete.

The antihistamine fexofenadine was identified as a substrate for P-glycoprotein-mediated efflux with use of polarized human intestinal cells and mice that lacked *mdr1a*-encoded P-glycoprotein.<sup>14</sup> Cellular fexofenadine uptake was also found to be mediated by human OATP and rat *oatp1* and *oatp2* with use of a recombinant vaccinia expression system.<sup>14</sup> Because the metabolism of fexofenadine is negligible in humans, P-glycoprotein and OATP transporters may be important determinants of fexofenadine disposition.

The purpose of this study was initially to examine the effect of grapefruit, orange, and apple juices and their constituents on P-glycoprotein and OATP transporter activities in vitro. The clinical relevance of these findings was subsequently assessed in a study that evaluated the influence of juices from these fruits on the disposition of fexofenadine in humans. The results suggest a new model for intestinal drug absorption and mechanism of food-drug interaction.

## METHODS

### In vitro drug transport studies

**Juice, segments, extract, and constituents.** Grapefruit juice (Minute Maid Premium frozen concentrated grapefruit juice, 100% pure; Minute Maid Company, Houston, Tex), orange juice (Minute Maid Premium frozen concentrated orange juice, 100% pure, pulp free; Minute Maid Company), and apple juice (Seneca frozen concentrated apple juice; Seneca Foods Corporation, Marion, NY) were purchased from Compton's Foodmart, Nashville, Tenn. The same brand and lot numbers of each were used throughout the in vitro studies.

Grapefruit segments and extracts were prepared from Duncan seedy white grapefruit (Ocean Spray, Indian River, Fla). They had been tested previously in a clinical study and were found to increase the oral bioavailability of felodipine by inhibition of intestine CYP3A4 activity.<sup>15</sup> To prepare the segments, whole white grapefruits were grated to remove the flavedo (yellow outer layer). The albedo (whitish spongy layer), vascular elements surrounding the segments, and core were removed by hand. The cleaned individual segments that contained seeds were combined and homogenized in a blender. To prepare the grapefruit extract, the segment-free parts (flavedo, albedo, vascular elements, and core) were extracted with absolute ethanol, filtered, and evaporated to an aqueous-based concentrated solution that contained approximately 5% ethanol, as described previously.<sup>15</sup> Both grapefruit segments and extracts were stored frozen at  $-80^{\circ}\text{C}$  until use.

Bergamottin was purchased from Indofine Chemical Co (Somerville, NJ). 6',7'-Dihydroxybergamottin was supplied by Drs Tony Montanari and Bill Widmer, Florida Department of Citrus, Lake Alfred, Fla. All other constituents were purchased from Sigma Chemical Co (St Louis, Mo) and were the highest grade available.

**Determination of P-glycoprotein activity.** Three polarized epithelial cell lines that expressed different content of human P-glycoprotein were used. Caco-2, LLC-PK1, and L-MDR1 cell lines were cultured on polycarbonate membrane filters (Transwell filters, Costar Corp, Cambridge, Mass) as described previously.<sup>7</sup> Transport was determined by addition of [<sup>3</sup>H]digoxin (19 Ci/mmol; DuPont-New England Nuclear, Boston, Mass) or [<sup>3</sup>H]vinblastine (11.1 Ci/mmol; Amersham Life Science, Arlington Heights, Ill) to either the basal or the apical side of the polarized cell monolayer and subsequent periodic measurement of accumulation of radioactivity in the other

compartment over 4 hours.<sup>7</sup> Epithelialized Caco-2 cells that expressed high levels of P-glycoprotein were used to model intestinal transport. LLC-PK1 cells (negative control) and stably transfected L-MDR1 cells that expressed very high levels of P-glycoprotein (positive control) were also tested. Much greater basal-apical transport of digoxin and vinblastine was observed compared with apical-basal movement during baseline conditions in Caco-2 and L-MDR1 cells. Net efflux transport of digoxin and vinblastine was not observed during baseline conditions in LLC-PK1 cells. Potential modulators of P-glycoprotein activity were added to both compartments. Cellular integrity and paracellular leakage were monitored by inulin flux across the monolayer. Grapefruit, orange, and apple juice concentrations up to 5% of normal strength were associated with less than 1% per hour of basal-apical or apical-basal flux of inulin. However, greater concentrations of the juices produced increasing flux of inulin indicative of cytotoxicity, and they were not tested.

**Determination of OATP and sodium-taurocholate cotransporting polypeptide (NTCP) activities.** HeLa cells grown in 12-well plates (approximately  $0.8 \times 10^6$  cells/well) were infected with vaccinia at a multiplicity of infection of 10 plaque-forming units (pfu)/cell in serum-free Optimem I medium (Invitrogen Life Technologies, Carlsbad, Calif) and allowed to adsorb for 30 minutes at 37°C. Cells in each well were then transfected with 1 µg of wild-type or uptake transporter complementary deoxyribonucleic acid (cDNA), along with Lipofectin reagent (Invitrogen Life Technologies) and incubated at 37°C for 16 hours. The parental plasmid without any insert was used as control. Transport was then evaluated as outlined previously.<sup>14</sup> [<sup>14</sup>C]-Fexofenadine and [<sup>3</sup>H]-sodium taurocholate were the substrates for OATPs and NTCP transporters, respectively. To measure the fexofenadine transport kinetics, [<sup>14</sup>C]-fexofenadine uptake during the linear phase (first 5 minutes) was assessed in the presence of varying concentrations of unlabeled fexofenadine. Passive diffusion was determined by performing parallel experiments with use of the parental plasmid DNA without the transporter cDNA, and this value was then subtracted from the total uptake rate observed in the presence of the transporter cDNA. Michaelis-Menten type nonlinear curve-fitting was carried out to obtain estimates of the maximal uptake rate ( $V_{max}$ ) and the concentration at which half the  $V_{max}$  occurs (Michaelis-Menten constant [ $K_m$ ]) (GraphPad Prism, GraphPad Software Inc, San Diego, Calif). The 50% inhibitory

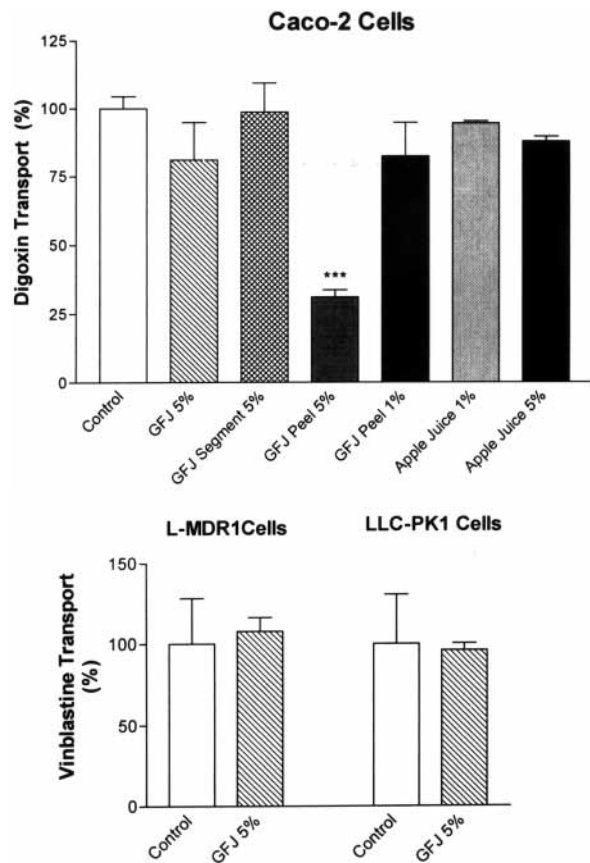
concentration ( $IC_{50}$ ) values were estimated from the Hill equation. All experiments were executed twice on at least 2 to 3 separate experimental days.

### Human volunteer study

**Study population.** Six men (age range, 19-40 years) and 4 women (age range, 19-28 years) were tested. An evaluation before the study showed that subjects had normal findings on physical examination and on laboratory testing, which included routine hematologic and serum chemical testing. No subject had any significant illnesses within the preceding 2 weeks, had routinely used medications, or had a history of cardiac, renal, hepatic, or gastrointestinal disease or of drug or alcohol abuse. All individuals provided written informed consent for the study, which had been approved by the Health Sciences Standing Committee on Human Research at the University of Western Ontario (London, Ontario, Canada).

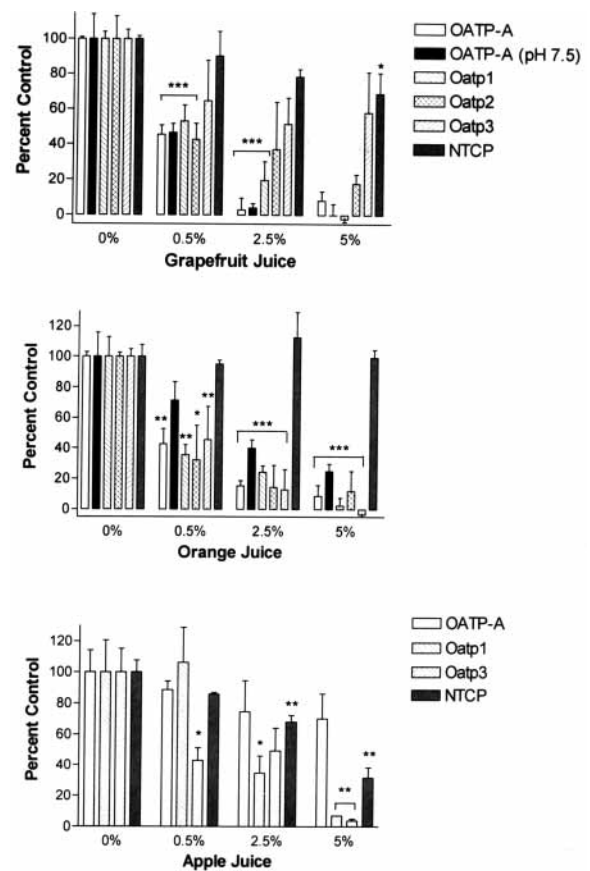
**Experimental protocol.** Subjects received 2 fexofenadine tablets (60 mg each; Allegra; Hoechst Marion Roussel Canada Inc, Laval, Quebec, Canada) with grapefruit juice (2 concentrations), orange juice, apple juice, or water in a single-dose, balanced, randomized, 5-way crossover study. All juices were purchased from the same supplier (Everfresh, Windsor, Ontario, Canada) at a normal strength that had been previously reconstituted from concentrate. The volume and schedule of fluid (juice or water) administration were as follows: 300 ml with 2 fexofenadine tablets, followed by 150 ml every 0.5 to 3.0 hours (total volume, 1.2 L). All juices were given at normal strength except for low-dose grapefruit juice, which was administered at 25% of regular strength (equivalent to 1 glass of grapefruit juice). Peripheral venous blood (5 ml) was sampled into tubes that contained ethylenediaminetetraacetic acid just before and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, and 8.0 hours after administration for the purpose of measuring plasma fexofenadine concentrations. Urine was collected between 0 and 24 hours to determine the pharmacokinetics of fexofenadine renal elimination. Subjects fasted for 10 hours before testing. They consumed a standardized lunch after 4 hours (noon). Smoking and consumption of beverages that contained caffeine were not allowed during testing. Medications (including over-the-counter drugs) and grapefruit, orange, and apple juices other than those provided in the study were not permitted 1 week before or during the study. The washout interval among study days was 1 week.

**Assay of fexofenadine.** A 100-mg  $C_{18}$  preparatory solid-phase extraction column (Sep-Pak Vac cartridge,



**Fig 1.** Effect of grapefruit juice (GFJ), segment, and peel and apple juice on P-glycoprotein-mediated [ $^3\text{H}$ ]digoxin (5  $\mu\text{mol/L}$ ) or [ $^3\text{H}$ ]vinblastine (5  $\mu\text{mol/L}$ ,  $n = 3$ ) efflux transport in Caco-2, L-MDR1, and LLC-PK1 cells. Three asterisks,  $P < .001$ .

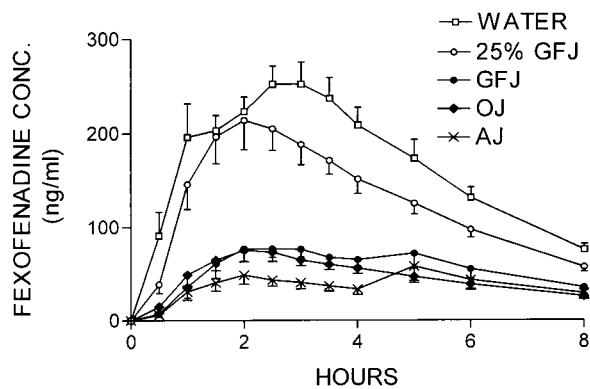
Waters Corp, Mississauga, Ontario, Canada) was initially washed with 1-ml volumes of isopropyl alcohol, methanol, and water. A 500- $\mu\text{l}$  aliquot of plasma or urine was then added, and the column was washed with 1-ml volumes of water and methanol/water (20:80 [vol/vol]). The sample was eluted with 1 ml methanol that contained triethylamine (200  $\mu\text{l}/100 \text{ ml}$ ), and it was evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved in a 100- $\mu\text{l}$  aliquot of HPLC mobile phase that consisted of acetonitrile/water (40:60 [vol/vol]) and 1 ml/L triethylamine (pH 3.0) with phosphoric acid that also contained the internal standard diphenhydramine (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada) at a concentration of 500 ng/ml. The solution was filtered (0.45  $\mu\text{m}$ ),



**Fig 2.** Inhibition of human OATP and rat oatp and NTCP by grapefruit, orange, and apple juice. The oatp-mediated [ $^{14}\text{C}$ ]fexofenadine uptake (1  $\mu\text{mol/L}$ ) or [ $^3\text{H}$ ]taurocholate (5  $\mu\text{mol/L}$ ) was assessed in the presence or absence of increasing concentrations of fruit juices after 10 minutes in HeLa cells ( $n = 4$  to 6). One asterisk,  $P < .05$ ; 2 asterisks,  $P < .01$ ; 3 asterisks,  $P < .001$ .

and a sample (30  $\mu\text{l}$ ) was injected onto a Spherisorb C<sub>8</sub> 5- $\mu\text{m}$  column (75 mm  $\times$  3.2 mm; Phase Separation Ltd, Deeside, Clwyd, United Kingdom) with a mobile flow rate of 0.4 ml/min. Fluorescence detection (excitation wavelength, 223 nm; emission wavelength, 290 nm) was used to monitor the effluent. The retention times of diphenhydramine and fexofenadine were 6.5 and 10.9 minutes, respectively. The standard curve of fexofenadine was linear over the range tested (0-400 ng/ml). The coefficient of variation was 4.8% at 25 ng/ml ( $n = 5$ ), and the limit of detection was 5 ng/ml.

**Data analysis.** Plasma fexofenadine concentrations were analyzed by use of a noncompartmental model method. The terminal elimination rate constant ( $k_e$ ) was determined by log-linear regression of the final data



**Fig 3.** Mean plasma fexofenadine concentration–time profiles for persons ( $n = 10$ ) orally administered fexofenadine (120 mg) with 300 ml water, grapefruit juice at 25% of regular strength (25% GFJ), grapefruit juice (GFJ), orange juice (OJ), or apple juice (AJ) followed by 150 ml of the same fluid every 0.5 to 3 hours (total volume, 1.2 L).

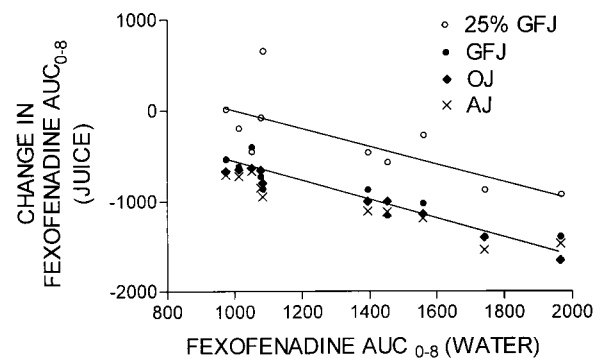
points (at least 3). The apparent elimination half-life of the log-linear phase ( $t_{1/2}$ ) was calculated as follows:  $0.693/k_e$ . The area under the plasma drug concentration–time profile from 0 to 8 hours [AUC(0-8)] was calculated by use of the linear trapezoidal method. The AUC from 8 hours to infinity [AUC(8- $\infty$ )] was determined by dividing the final plasma fexofenadine concentration by  $k_e$ . Peak plasma drug concentration ( $C_{max}$ ) and the time to reach  $C_{max}$  ( $t_{max}$ ) were obtained directly from the experimental data. Urinary excretion of fexofenadine from 0 to 24 hours ( $A_e$ ) was calculated as urinary concentration times volume. The renal clearance of fexofenadine was determined as follows:  $A_e/AUC(0-\infty)$ .

Descriptive and comparative statistics were calculated with use of SigmaStat version 1.0 (Jandel Scientific Software, San Rafael, Calif). Comparisons among the 5 groups initially used ANOVA for repeated measures. For those analyses in which  $P < .05$ , a priori comparisons were performed between water and the other treatments with the Bonferroni method for pairwise multiple comparison procedure. Data are presented as mean values  $\pm$  SE.

## RESULTS

### In vitro drug transport studies

**Effect on P-glycoprotein activity.** Concentrations of grapefruit juice and homogenized segments and apple juice that did not cause loss of cellular integrity or paracellular leakage (up to 5% of normal strength) did not alter basal-apical or apical-basal transport of digoxin



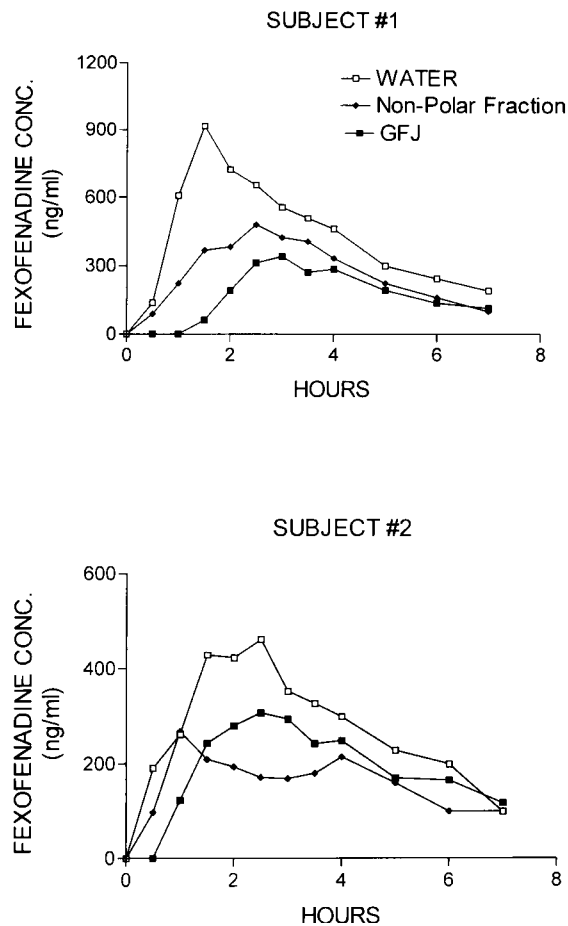
**Fig 4.** Change in area under the plasma fexofenadine concentration–time profile from 0 to 8 hours [AUC(0-8)] with 25% regular-strength grapefruit juice (25% GFJ), grapefruit juice (GFJ), orange juice (OJ), or apple juice (AJ) plotted against plasma fexofenadine AUC(0-8) with water for each individual ( $n = 10$ ). Fexofenadine (120 mg) was administered orally with 300 ml water or juice followed by 150 ml of the same fluid every 0.5 to 3 hours (total volume, 1.2 L).

or vinblastine in monolayers of Caco-2 or L-MDR1 cells (positive controls) or LLC-PK1 cells (negative control, Fig 1). However, extract of grapefruit peel at 5% of the original concentration reduced the net efflux transport in the Caco-2 cell line.

Isolated chemical components from grapefruit were tested for effect on P-glycoprotein activity. 6',7'-Dihydroxybergamottin was the most potent inhibitor ( $IC_{50}$ , 33  $\mu$ mol/L). Bergamottin did not alter P-glycoprotein activity at concentrations up to 50  $\mu$ mol/L. Naringin at 3000  $\mu$ mol/L reduced P-glycoprotein to 51%  $\pm$  9% of control ( $P < .001$ ).

**Effect on OATP and NTCP.** Grapefruit, orange, and apple juices inhibited the uptake of fexofenadine in a concentration-dependent manner by an array of human OATP and rat oatp transporters (Fig 2). In particular, grapefruit and orange juices at 5% of normal strength potently inhibited human OATP-A at an unbuffered and physiologic pH and at least 2 of 3 rat oatp transporters. Grapefruit and orange juices did not alter the uptake of sodium taurocholate by NTCP at the maximal tested concentration, whereas apple juice produced moderate inhibition.

Several citrus furanocoumarins and bioflavonoids, as well as their aglycones, were potent inhibitors of rat oatp3 activity (Table I). 6',7'-Dihydroxybergamottin was also a potent inhibitor of rat oatp1 ( $IC_{50}$ , 0.28  $\mu$ mol/L). Bergamottin and 5-methoxypsoralen caused little inhibition of rat oatp1.



**Fig 5.** Plasma fexofenadine concentration–time profiles for 2 subjects after oral administration of 120 mg fexofenadine with 300 ml water, nonpolar fraction of grapefruit juice, or grapefruit juice (GFJ).

### Human volunteer study

**Effect on fexofenadine pharmacokinetics.** Grapefruit, orange, and apple juices produced markedly lower plasma fexofenadine concentrations compared with water (Fig 3). Fexofenadine AUC and  $C_{max}$  values were decreased to 30% to 40% of those with water (Table II). Fexofenadine  $t_{max}$  and  $t_{1/2}$  were not different among treatments. Grapefruit juice at 25% of normal strength produced fexofenadine AUC and  $C_{max}$  values that were 80% of those observed with water.

The magnitude of decrease in fexofenadine AUC was variable among individuals (Fig 4). It was dependent on (1) baseline fexofenadine AUC (individuals with the highest fexofenadine AUC with water had the greatest decrease with the juice) and (2) juice concentration (normal grapefruit juice produced a greater decrease in

**Table I.** Effect of citrus furanocoumarins and bioflavonoids on uptake of fexofenadine by rat *oatp3*

	<i>oatp3</i> activity (% of control)	
	5 $\mu$ mol/L	50 $\mu$ mol/L
Furanocoumarins		
6',7'-Dihydroxybergamottin	24 $\pm$ 4*	22 $\pm$ 10*
Bergamottin	41 $\pm$ 10†	22 $\pm$ 11†
5-Methoxypsoralen	47 $\pm$ 5*	32 $\pm$ 8*
Bioflavonoids		
Glycosides		
Naringin	36 $\pm$ 8†	44 $\pm$ 18
Hesperidin	83 $\pm$ 7	51 $\pm$ 5*
Aglycones		
Naringenin	36 $\pm$ 7*	10 $\pm$ 11*
Hesperitin	40 $\pm$ 12‡	77 $\pm$ 25
Quercetin	52 $\pm$ 15‡	60 $\pm$ 24

\* $P < .001$ .

† $P < .01$ .

‡ $P < .05$ .

fexofenadine AUC than grapefruit juice at 25% of normal strength).

Grapefruit, orange, and apple juices reduced urinary excretion of fexofenadine to 30% of that with water; grapefruit juice at 25% of normal strength decreased urinary excretion of fexofenadine to 79% of that with water. None of the fruit juices altered renal clearance or urine volume.

### DISCUSSION

Transporters have been increasingly recognized as important determinants of drug disposition and resulting clinical response. The best-characterized drug transporter is P-glycoprotein. It transports numerous structurally and therapeutically unrelated drugs, including cyclosporine (INN, ciclosporin), digoxin, erythromycin, lovastatin, loperamide, HIV-protease inhibitors, and many chemotherapeutic agents.<sup>16</sup> P-glycoprotein is located on the luminal surface of the epithelial cells of the small intestine, the bile canalicular membrane of the liver, and the proximal tubule of the kidney and of endothelial cells that comprise the blood-brain and blood-testes barriers.<sup>17,18</sup> P-glycoprotein affects the disposition of drugs by limiting their absorption from the gut, by facilitating their removal by secretion into bile and urine, and by reducing their entry into brain and testes.

Grapefruit juice was originally shown to augment the oral bioavailability of the calcium channel blockers felodipine, and nifedipine.<sup>8</sup> In that case, the effect of

**Table II.** Plasma and urinary pharmacokinetics of 120 mg fexofenadine with 300 ml water, 25% regular-strength grapefruit juice, grapefruit juice, orange juice, or apple juice followed by 150 ml of the same fluid every 0.5 to 3.0 hours (total volume, 1.2 L)

	Water	25% Grapefruit juice	Grapefruit juice	Orange juice	Apple juice
Plasma fexofenadine					
AUC(0-8) (ng · h/ml)	1330 ± 109	1021 ± 98*	439 ± 44**	373 ± 19**	301 ± 33**
AUC(0-∞) (ng · h/ml)	1616 ± 120	1244 ± 111*	593 ± 67**	494 ± 16**	434 ± 53**
C <sub>max</sub> (ng/ml)	288 ± 23	228 ± 28	110 ± 14**	96 ± 7**	81 ± 13**
t <sub>max</sub> (h)	2.4 ± 0.2	2.6 ± 0.2	3.2 ± 0.4	2.7 ± 0.5	3.1 ± 0.5
t <sub>1/2</sub> (h)	2.6 ± 0.2	2.7 ± 0.2	3.1 ± 0.2	3.4 ± 0.3	3.5 ± 0.4
Urinary fexofenadine					
Ae(0-24) (mg)	7.7 ± 0.8	6.1 ± 0.5	2.5 ± 0.2*	2.5 ± 0.3*	2.4 ± 0.3*
CL <sub>R</sub> (0-24) (ml/ml)	78 ± 8	85 ± 7	74 ± 7	86 ± 8	92 ± 9
V(0-24) (L)	2.6 ± 0.2	2.6 ± 0.2	2.1 ± 0.3	2.2 ± 0.2	2.2 ± 0.3

Data are expressed as mean values ± SEM. AUC(0-8), Area under the plasma drug concentration–time curve from 0 to 8 hours; AUC(0-∞), plasma AUC from 0 hours extrapolated to infinity; C<sub>max</sub>, peak plasma drug concentration; t<sub>max</sub>, time to reach C<sub>max</sub>; t<sub>1/2</sub>, elimination half-life; Ae(0-24), urinary excretion of fexofenadine from 0 to 24 hours; CL<sub>R</sub>(0-24), renal clearance from 0 to 24 hours; V(0-24), volume of urine from time zero to 24 hours.

\*Difference between juice and water treatments, *P* < .05.

\*\*Difference between juice and water treatments, *P* < .001.

grapefruit juice almost certainly resulted from irreversible inactivation followed by proteolysis of intestinal CYP3A4.<sup>9</sup> Grapefruit juice has subsequently been shown to interact with more than 20 medications.<sup>10</sup> Another food, Seville (sour) orange juice, was recently found to interact with felodipine by the same mechanism as grapefruit juice.<sup>19</sup> However, Seville orange juice did not augment the oral bioavailability of the immunosuppressive agent cyclosporine despite marked reduction of enterocyte CYP3A4 content, whereas grapefruit juice did.<sup>20</sup> Therefore inactivation of intestinal CYP3A4 cannot be the only mechanism of action of grapefruit juice. Because cyclosporine is also a substrate for P-glycoprotein, grapefruit juice may also reduce the activity of this transporter. Furthermore, grapefruit juice increased the systemic availability of saquinavir, another substrate for both CYP3A4 and P-glycoprotein.<sup>21</sup> Therefore dietary constituents such as fruit juices may affect CYP enzymes and may potentially alter the activity of transporters such as P-glycoprotein.

Our in vitro work showed that grapefruit juice, homogenized unprocessed segments, and apple juice at concentrations up to 5% of normal strength did not affect P-glycoprotein-mediated drug transport. In contrast, 5% extract from mainly grapefruit peel decreased P-glycoprotein activity. Constituents in grapefruit are therefore able to inhibit in vitro P-glycoprotein-mediated net efflux when present in sufficient concentration. Although this conclusion supports that of Takanaga et al,<sup>12</sup> their interpretation was based on indirect measurements of P-glycoprotein transport with grapefruit juice concentrations up to 50% of normal

strength, which may have affected cell integrity. Our results are not consistent with those of Soldner et al,<sup>13</sup> who initially reported that grapefruit juice activated P-glycoprotein. However, this group later indicated that their findings may have been the result of equipment-generated artifacts.<sup>19</sup>

Two constituents in grapefruit, 6',7'-dihydroxybergamottin at 33 μmol/L and naringin at 3000 μmol/L, were shown to reduce in vitro P-glycoprotein activity by half. 6',7'-Dihydroxybergamottin has been previously reported not to reduce P-glycoprotein activity. However, this conclusion was based on results with use of either inadequate 6',7'-dihydroxybergamottin concentrations (5 and 10 μmol/L),<sup>20</sup> indirect measurement of P-glycoprotein transport (intracellular accumulation of a fluorescent metabolite formed in the cytosol from calcein, a P-glycoprotein substrate),<sup>20</sup> or a cell monolayer that appeared to have compromised integrity because of increased apical to basal flux of 6',7'-dihydroxybergamottin at the high concentration tested (200 μmol/L).<sup>22</sup> Because we measured 6',7'-dihydroxybergamottin concentrations at 42 μmol/L in commercial juice and at 118 μmol/L in segments from grapefruit,<sup>15</sup> this substance consumed in either juice or segments may have some inhibitory effect on P-glycoprotein activity in vivo. Naringin concentration in commercial grapefruit juice is about 750 μmol/L<sup>15</sup>; therefore this constituent in commercial juice is not likely to produce relevant in vivo inhibition of P-glycoprotein activity. However, naringin concentrations in segments and extract from whole grapefruit were measured at 2300 μmol/L and 33,100 μmol/L, respectively. Naringin in grapefruit segments may therefore be sufficient to cause

some inhibition of P-glycoprotein activity in humans. In addition, the high concentration of naringin in the extract could account for inhibition of *in vitro* P-glycoprotein activity after the extract was diluted to 5% of its original concentration.

Because P-glycoprotein substrates such as fexofenadine are also substrates of OATP uptake transporters<sup>14</sup> and because members of the OATP transporters have been shown to be capable of mediating the cellular uptake of other structurally divergent drugs such as digoxin and pravastatin,<sup>16</sup> it was necessary to delineate the relative contribution of both P-glycoprotein and uptake transporters to understand more fully the effect of grapefruit and other fruit juices. Accordingly, *in vitro* studies with grapefruit, orange, and apple juices and constituents as modulators of OATP-mediated fexofenadine uptake were pursued. Grapefruit juice potently inhibited OATP-mediated fexofenadine uptake by human and rat transporters in a dose-dependent fashion. A grapefruit juice concentration at only 0.5% of normal strength decreased the activity by half. This contrasts with the effect of grapefruit juice at a 10-fold higher concentration, which did not alter P-glycoprotein efflux or bile acid uptake transporter activities. Orange juice produced results nearly identical to those for grapefruit juice. Apple juice also reduced the activity of OATP transporters, but it appeared to differ in the spectrum of uptake transporters affected. Therefore several fruit juices produced relatively more potent inhibition of several OATP transporters than of P-glycoprotein.

6',7'-Dihydroxybergamottin, bergamottin, and naringin at a concentration of 5  $\mu\text{mol/L}$ , which is 8-, 5-, and 150-fold, respectively, less than the concentration measured in normal-strength grapefruit juice,<sup>15</sup> produced greater than 50% inhibition of oatp3 activity. 5-Methoxypsoralen at 5  $\mu\text{mol/L}$ , which was 6-fold less than that found in Seville (sour) orange juice,<sup>19</sup> and hesperidin at 50  $\mu\text{mol/L}$ , which was 2-fold less than that found in Valencia (sweet) orange juice,<sup>23</sup> decreased oatp3 activity by half. Because rat oatp3 is expressed on the brush-border of intestinal enterocytes, inhibition of this uptake transporter by these substances may produce important reduction of oral drug absorption.<sup>24</sup> Additional studies with another uptake transporter revealed that 6',7'-dihydroxybergamottin had an estimated  $\text{IC}_{50}$  of 0.28  $\mu\text{mol/L}$  for oatp1. When this was compared with the  $\text{IC}_{50}$  of 33  $\mu\text{mol/L}$  for P-glycoprotein inhibition, it was apparent that 6',7'-dihydroxybergamottin had more than a 2-log greater order of magnitude for inhibition of oatp1 than that of P-glycoprotein. Therefore several furanocoumarins and bioflavonoids are potent inhibitors of different types of

rat oatp. This may explain the common inhibitory effect by a number of fruit juices. Moreover, these substances may be present in sufficient concentration in these juices to produce more pronounced *in vivo* inhibition of OATPs than of P-glycoprotein.

Uptake and efflux transporters can be expressed in the same cell.<sup>16</sup> In the intestine both OATP and P-glycoprotein transporters are located on the luminal membrane of the enterocyte, which results in opposing vectors for uptake into the portal circulation and for efflux back into the bowel. In the liver the location of OATPs on the basolateral membrane and P-glycoprotein on the bile canalicular membrane of hepatocytes facilitates the efficient unidirectional movement of drugs from portal circulation into bile for secretion back into the intestine. The OATP and P-glycoprotein substrate fexofenadine has an absolute oral bioavailability estimated at 33% in humans (product information, Hoechst Marion Roussel, Laval, Quebec, Canada). It is eliminated from the body unchanged, mainly by biliary and gastrointestinal secretion in feces, with much less by excretion in urine.<sup>25</sup> Therefore the oral absorption and elimination of fexofenadine in humans appears to be largely dependent on OATP and P-glycoprotein transporter activities found in the gastrointestinal tract and liver.

The marked decrease in plasma fexofenadine AUC and  $C_{\text{max}}$  values with no change in  $t_{\text{max}}$  and  $t_{1/2}$  by grapefruit, orange, and apple juices was consistent with reduced bioavailability as opposed to enhanced systemic elimination. The lack of change in the renal clearance of fexofenadine further supports this conclusion. The active constituents in the fruit juices likely would be at higher concentrations in the small intestine than in the liver. Moreover, inhibition of drug uptake at the liver immediately after drug absorption (first-pass effect) would act to increase, rather than decrease, plasma fexofenadine concentrations. Consequently, the primary mechanism of the interaction most likely resulted from inhibition of a process that is important for the absorption of fexofenadine from the gastrointestinal tract.

Fexofenadine AUC values during baseline conditions varied among individuals. It might be expected that those subjects with a high fexofenadine AUC value would have relatively high intestinal OATP activity. There was also a high correlation between baseline fexofenadine AUC and magnitude of decrease with juice and strength of juice among individuals. Inasmuch as elevated enzyme or transporter activity is generally more sensitive to inhibition, these findings suggest that grapefruit, orange, and apple juices acted by preferentially reducing intestinal OATP function.

Although a direct inhibitory effect by fruit juices on intestinal OATP transporters may explain the interaction observed in humans, other mechanisms need to be considered. If water were absorbed from the gut faster when administered alone than in fruit juice, it may produce higher fexofenadine concentration and slower drug transport in the intestine. This could cause higher drug exposure to OATP transporters and result in greater drug absorption. Another mechanism for decreased fexofenadine bioavailability by fruit juices could therefore involve an indirect effect on OATP function from enhanced intestinal fluid volume by the nonspecific osmotic effects of solutes. In an attempt to assess the possible importance of direct inhibition of OATP function in humans, a pilot project was conducted. Grapefruit juice was processed by use of solid-phase column chromatography to obtain a nonpolar fraction that contained specific constituents that were shown to inhibit *in vitro* OATP activity but not substances that contributed to the majority of the osmotic property of the juice. The effects on fexofenadine bioavailability by 300 ml grapefruit juice, water, and a nonpolar fraction reconstituted in water were compared (Fig 5). This volume of grapefruit juice produced lower plasma fexofenadine concentrations than those with water in 2 volunteers, suggesting that this interaction occurred with a more normal amount of juice. Clinically, this may mean a reduced effectiveness of fexofenadine. The nonpolar fraction also caused lower fexofenadine concentrations compared with water. Direct inhibition by specific constituents in grapefruit, and possibly other juices, may therefore be a mechanism that explains at least part of the interaction.

In conclusion, the results of this study provide support for a new model that describes the possible interplay between drug-metabolizing enzymes and transporters that ultimately determine drug disposition and the resulting clinical response. This model now includes the role of drug uptake by OATP transporters in addition to drug metabolism by CYP3A4 and efflux transport by P-glycoprotein. We have shown for the first time that fruit juices and constituents can interact with members of the OATP transporter family by reducing their activities. The functional consequences of such an interaction are reflected as a significant reduction in the oral bioavailability of fexofenadine, possibly by preferential direct inhibition of intestinal OATP activity. This interaction is likely relevant to other drugs and may explain, at least partially, the lack of bioequivalence and subsequent withdrawal from the market of a formulation of cyclosporine when mixed with apple juice.<sup>26</sup> The results of this study suggest a new mechanism to account for certain food-drug interactions.

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