

A Modified Grapefruit Juice Eliminates Two Compound Classes as Major Mediators of the Grapefruit Juice–Fexofenadine Interaction: An In Vitro–In Vivo “Connect”

By: Christina S. Won, Tian Lan, Karen M. VanderMolen, Paul a, Dawson, [Nicholas H. Oberlies](#), Wilbur W. Widmer, Yolanda V. Scarlett, Mary F. Paine

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Abstract:

The grapefruit juice (GFJ)–fexofenadine interaction involves inhibition of intestinal organic anion transporting polypeptide (OATP)-mediated uptake. Only naringin has been shown clinically to inhibit intestinal OATP; other constituents have not been evaluated. The effects of a modified GFJ devoid of furanocoumarins (~99%) and polymethoxyflavones (~90%) on fexofenadine disposition were compared to effects of the original juice. Extracts of both juices inhibited estrone 3-sulfate and fexofenadine uptake by similar extents in OATP-transfected cells (~50% and ~25%, respectively). Healthy volunteers (n = 18) were administered fexofenadine (120 mg) with water, GFJ, or modified GFJ (240 mL) by randomized, three-way crossover design. Compared to water, both juices decreased fexofenadine geometric mean AUC and C_{max} by ~25% (P ≤ .008 and P ≤ .011, respectively), with no effect on terminal half-life (P = .11). Similar effects by both juices on fexofenadine pharmacokinetics indicate furanocoumarins and polymethoxyflavones are not major mediators of the GFJ–fexofenadine interaction.

Keywords: clinical pharmacology | clinical research | gastrointestinal | pharmacokinetics | drug metabolism

Article:

The drug interaction liability of grapefruit juice (GFJ) and individual constituents has been studied extensively for more than 20 years.^[1] GFJ–drug interactions can manifest clinically as increased systemic drug exposure and subsequent adverse reactions due to mechanism-based inhibition of cytochrome P450 3A (CYP3A)-mediated metabolism in the intestine by GFJ.^[2, 3] A

class of constituents, furanocoumarins, was established as major mediators of this effect.^[3-5] A different type of GFJ interaction was reported in 2002, when the non-sedating, minimally metabolized antihistamine fexofenadine was used as a probe substrate for examining the effect of GFJ on the efflux transporter, P-glycoprotein (P-gp), in the intestine.^[6] Healthy volunteers administered fexofenadine with GFJ exhibited an unexpected mean 63% decrease in fexofenadine exposure. This seemingly paradoxical effect was observed consistently in four independent clinical studies reported over the subsequent five years.^[7-10] The underlying mechanism was postulated to involve inhibition of fexofenadine active uptake in the intestine by organic anion transporting polypeptides (OATPs). Three clinical studies demonstrated that the size and flare of histamine-induced skin wheals were increased after administration of fexofenadine with GFJ or orange juice but not water.^[11] Population pharmacokinetic analysis of the combined data from these studies and a bioequivalence study showed that the oral availability of fexofenadine was reduced by 36%. Due to the potential for reduced therapeutic efficacy, the labeling of all fexofenadine products recommends taking the drug with water.

Constituents in GFJ have been identified as OATP inhibitors *in vitro*,^[10, 12] but the flavanone, naringin, is the only single constituent tested clinically.^[10] Relative to water, GFJ and an aqueous solution of naringin at the same concentration as that in GFJ (~1,200 µmol/L) decreased fexofenadine mean exposure by 42% and 22%, respectively. A suspension of a particulate fraction of GFJ containing three one-hundredths the concentration of naringin (34 µmol/L) had no effect on fexofenadine exposure. The investigators concluded that naringin was a major causative ingredient inhibiting enteric OATP. However, the ~50% difference in fexofenadine exposure between GFJ and naringin suggests other constituents contributed to the interaction.

The furanocoumarins, bergamottin and 6',7'-dihydroxybergamottin (DHB), and the polymethoxyflavones, tangeretin and nobiletin, have been reported to inhibit human enteric OATP activity *in vitro*.^[12] Whether these observations translate to the clinic is not known. Based on the cumulative data, these two classes of GFJ constituents were evaluated as candidate OATP inhibitors by comparing the effect of a GFJ devoid of furanocoumarins and polymethoxyflavones (i.e., modified GFJ, mGFJ) with that of the original GFJ on fexofenadine disposition in both OATP-transfected cells and healthy volunteers.

Methods

Materials and Chemicals

[³H]estrone 3-sulfate (E1S) ammonium salt (54.3 Ci/mmol) was purchased from Perkin Elmer (Waltham, MA). [³H]fexofenadine (6 Ci/mmol), originally a gift from GlaxoSmithKline (Research Triangle Park, NC) and custom synthesized by Amersham Life Sciences (Piscataway, NJ), was provided by Dr. Dhiren Thakker (Eshelman School of Pharmacy, Chapel Hill, NC). E1S potassium salt, verapamil (VER), bromosulfophthalein (BSP), bergamottin, DHB, naringin, hesperidin, tangeretin, and nobiletin were purchased from Sigma-Aldrich (St. Louis, MO).

Fexofenadine was obtained from Tocris Bioscience (Minneapolis, MN). COS-1 and HEK293T/17 cells were obtained from American Type Culture Collection (Manassas, VA). Cell culture plates were purchased from Corning Life Sciences (Tewksbury, MA). Opti-MEM and Lipofectamine2000 were purchased from Invitrogen (Carlsbad, CA). X-tremeGENE 9 was purchased from Roche Applied Science (Indianapolis, IN). Human OATP1A2 (variant 1; accession number NM_134431.1) and OATP2B1 expression plasmids were obtained from Origene Technologies (Rockville, MD). Plasmids for mock-transfected cells, pEYFP-C1 and pcDNA3.1/Hygro(+), were purchased from Clontech (Mountain View, CA) and Invitrogen, respectively. MDCKII parental cells and stably transfected MDCKII–OATP2B1 cells were provided by Dr. Markus Grube (Ernst-Moritz-Arndt University, Greifswald, Germany). All other chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA).

Preparation of Whole and Modified Grapefruit Juice

A commercial GFJ concentrate was obtained from a Florida processing facility and prepared as described previously.^[5,13] The GFJ and mGFJ were re-pooled, re-pasteurized at 71.7°C for 6 seconds, poured into sterilized bottles, and stored at –20°C until use. Representative compounds from the furanocoumarin, polymethoxyflavone, and flavanone classes were re-measured by HPLC as described previously.^[5,13]

Preparation of Whole and Modified Grapefruit Juice Extracts

Concentrated extracts of GFJ and mGFJ were prepared by adding 20 mL ethyl acetate to a 50-mL conical polypropylene tube containing 25 mL juice. Contents were shaken vigorously for 30 seconds and centrifuged (2,500g for 30 minutes at 25°C). The resulting organic layer was transferred to a 250-mL round-bottom glass flask. The extraction procedure was repeated twice, with each resultant organic layer combined in the flask. Organic layers were evaporated in vacuo. The residue was transferred to a 2-mL vial using methanol as a rinse and evaporated to dryness under air. The dried material was resuspended with 500 µL methanol, yielding a 200-fold concentrated extract of the starting juice volume (100 mL).

Transient Transfection of OATP1A2 and OATP2B1 Into COS-1 and HEK293T/17 Cells

Human OATP1A2 and OATP2B1 expression plasmids were verified by DNA sequencing prior to use. COS-1 cells were maintained, seeded, and transfected transiently as described previously.^[14] HEK293T/17 cells were cultured and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C and 5% CO₂. Cells were seeded on Day 0 onto 100-mm dishes and transfected on Day 1 using the X-tremeGENE9 transfection reagent per manufacturer instructions. Transfection efficiency was assessed on Day 2 with fluorescence microscopy by estimating the percentage of cells expressing EYFP (mock). On Day 3, transfected cells were trypsinized, seeded onto 24-well plates, and incubated for approximately 48 hours before commencing the uptake assays. MDCKII parental cells and stably transfected MDCKII–OATP2B1 cells were cultured and maintained in a similar manner as

HEK293T/17 cells. Cells were passaged once, seeded onto 24-well plates, and incubated for approximately 48 hours prior to initiating uptake studies.

Uptake and Inhibition Assays

On Day 4 or 5, cells were washed and preincubated for 30 minutes at 37°C in uptake buffer (pH 6).^[14] Buffer was replaced, and cells were incubated with a dosing solution (200 µL) consisting of radiolabeled (plus unlabeled) E1S (total: 5 µmol/L) or fexofenadine (total: 5 µmol/L) in the presence of vehicle (1% methanol), VER (250 µmol/L) and/or BSP (250 µmol/L), or diluted juice extract (1:200 and 2:200 to approximate single- and double-strength concentrations, respectively, of the clinical juice). After 3 (E1S) or 30 (fexofenadine) min, cells were washed thrice with ice-cold phosphate-buffered saline and lysed with either 0.1 N sodium hydroxide (COS-1 cells) or 1% Triton X-100 in phosphate-buffered saline (HEK293T/17 cells). Liquid scintillation cocktail (5 mL) was added to 200-µL aliquots of the cell lysates, and radioactivity was counted. Protein concentrations were determined with a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Waltham, MA). Uptake was linear over the selected times for each substrate (data not shown).

Clinical Study Protocol

The study protocol was reviewed and approved by the University of North Carolina Biomedical Institutional Review Board and Clinical and Translational Research Center (CTRC) Committee.

Participants

All subjects provided written informed consent prior to participation. Healthy volunteers (nine women, nine men) were enrolled. The median age (range) of the women and men was 30 (23–54) and 37 (23–60) years, respectively. Participants were self-identified as Caucasian (seven women, five men), African-American (two women, two men), Asian (one man), or Hispanic (one man). Concomitant medications included oral/vaginal ring contraceptive therapy (two Caucasian women), hydrochlorothiazide (one Caucasian woman), low-dose aspirin (one African-American man), multivitamin, calcium, folate, and ω-3 fatty acid supplements (three women, three men). Prior to enrollment, each volunteer underwent a medical history, physical examination, and laboratory tests (i.e., liver function tests, basic metabolic panel, complete blood count). All women underwent a serum pregnancy test. Subjects were instructed to abstain from all fruit juices for ≥7 days prior to and during the study and to abstain from alcohol- and caffeine-containing beverages the evening before each admission. Each subject was assigned randomly to one of six treatment sequences: ABC, CAB, BCA, CBA, BAC, or ACB (A = water, B = GFJ, C = mGFJ).

Study Design

Eligible volunteers were admitted to the CTRC the evening prior to each of three study phases, which were separated by ≥ 10 days. All women underwent a repeat serum pregnancy test on the evening of each admission. Following an overnight fast beginning at midnight and placement of an indwelling venous catheter in an antecubital vein, each subject ingested two 60 mg fexofenadine tablets (Prasco Laboratories, Mason, OH) with 240 mL water, GFJ, or mGFJ. Blood (7 mL) was collected into EDTA-containing tubes (Becton-Dickinson, Franklin Lakes, NJ) before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 hours after fexofenadine administration. Subjects continued to fast until after the 4-hour blood collection, after which meals and snacks, devoid of fruit juices and caffeinated beverages, were provided. Vital signs were recorded at baseline and monitored periodically. Subjects were discharged after the 12-hour blood collection and returned for outpatient blood draws at 24, 36, 48, and 72 hours post-fexofenadine administration. Plasma was separated from blood cells by centrifugation within 1 hour of collection, transferred into cryovials, and stored at -80°C pending analysis for fexofenadine.

Analysis of Plasma for Fexofenadine

Plasma collections were processed by transferring 50 μL to a 96-well plate insert and precipitating proteins with 150 μL of methanol containing fexofenadine- d_6 (1 nmol/L) as internal standard (Toronto Research Chemicals, Inc., Toronto, ON, Canada). The mixtures were vortex-mixed for 5 minutes and centrifuged (3,000g for 10 minutes at 4°C). Calibration solutions (0.0012–7.2 $\mu\text{mol/L}$) and quality controls (0.005, 0.05, 0.1, 0.5, 1 $\mu\text{mol/L}$) were prepared similarly using fexofenadine and multiple-donor pooled plasma (Biological Specialty Corporation, Colmar, PA). Plasma was analyzed for fexofenadine by HPLC-tandem mass spectrometry using an API 4000 triple quadrupole with TurboIonSpray interface (Applied Biosystems/MDS Sciex, Concord, ON, Canada) as described.^[15] Briefly, 5 μL were injected, and fexofenadine and fexofenadine- d_6 were eluted from an Aquasil C18 column (2.1 mm \times 50 mm, particle diameter = 5 μm ; Thermo Fisher Scientific) using a mobile phase gradient (A: 0.1% formic acid in water; B: 0.1% formic acid in methanol) at a flow rate of 0.75 mL/min. The mass spectrometer was operated in positive-ion mode. Multiple reaction monitoring was used to detect fexofenadine (502 \rightarrow 466 m/z) and fexofenadine- d_6 (508 \rightarrow 472 m/z). The lower limit of quantification was 0.0012 $\mu\text{mol/L}$; inter- and intra-day coefficients of variation were 12% and $<15\%$, respectively, for the quality controls.

Data Analysis

OATP1A2- and OATP2B1-mediated uptake, in the absence or presence of inhibitor, was normalized for protein content. Net uptake was determined by subtracting the uptake in mock-transfected cells from that in OATP1A2- and OATP2B1-expressing cells incubated under parallel conditions.

Fexofenadine pharmacokinetics were evaluated by non-compartmental methods using WinNonlin (v 5.2, Pharsight Corp., Mountain View, CA). The terminal elimination rate constant

(λ_z) was estimated by log-linear regression of at least the last three data points in the terminal phase of the plasma concentration–time profile. The terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. The maximum concentration (C_{\max}), time to reach C_{\max} (t_{\max}), and the last measured concentration (C_{last}) were determined visually from the concentration–time profile. $AUC_{0\text{--last}}$ was calculated using the trapezoidal rule with linear up/log down interpolation. AUC from zero to infinite time ($AUC_{0\text{--}\infty}$) was calculated as the sum of AUC_{last} and $C_{\text{last}}/\lambda_z$. Apparent oral clearance (Cl/F) was calculated as Dose/ $AUC_{0\text{--}\infty}$. Below limit of quantification concentrations were excluded from data analysis.

Statistical Analysis

Statistical analysis employed SigmaPlot (v 11, Systat Software, Inc., San Jose, CA). In vitro data are presented as means \pm SDs of triplicate incubations. Two-way analysis of variance followed by Tukey's test was used to test for differences between vehicle and inhibitor treatments. Student's unpaired t -test was used to test for differences between mock- and OATP-transfected cells. A P -value $< .05$ was considered statistically significant. Pharmacokinetic outcomes ($t_{1/2}$, C_{\max} , $AUC_{0\text{--}\infty}$, Cl/F) are reported as geometric means with coefficients of variation (CV%). Medians and ranges are reported for t_{\max} . The Wilcoxon signed-rank test was used to compare t_{\max} . Pharmacokinetic outcomes, expressed as the ratio of GFJ to water or GFJ to mGFJ, are reported as geometric means with 90% confidence intervals. Comparisons using one-tailed paired Student's t -tests (given the uni-directional nature of the interaction) with Bonferroni correction (i.e., $0.05/2$) were used to detect differences between water and GFJ and between GFJ and mGFJ treatments. A P -value $< .025$ was considered statistically significant. Based on the method proposed by Lauzon and Caffo,^[16] for the primary pharmacokinetic measure of interest, AUC, a minimum of 18 subjects who completed all treatment phases was deemed adequate for this study, assuming a within-subject CV of 23% for fexofenadine determined from a previous study.^[10] The randomization scheme was generated using SAS (v 9.2, SAS Institute, Inc., Cary, NC).

Results

Representative Furanocoumarin, Polymethoxyflavone, and Flavanone Concentrations in Grapefruit Juice and Modified Grapefruit Juice

Due to the >5 -year lapse since last use,^[5,13] GFJ and mGFJ were re-analyzed for representative compounds from each of three phytochemical classes shown to inhibit intestinal OATP activity in vitro (Table 1). Relative to GFJ, the representative furanocoumarins DHB and bergamottin in mGFJ were reduced by $>99\%$ and 95% , respectively. The representative polymethoxyflavones nobiletin and tangeretin were reduced by 95% and 73% , respectively. The representative flavanones naringin, narirutin, and hesperidin were reduced by $\sim 30\%$, 32% , and 44% , respectively. Mean (\pm SD) aggregate representative furanocoumarins, polymethoxyflavones, and flavanones measured in GFJ (48 ± 1 , 0.48 ± 0.03 , and $1,064 \pm 10$ $\mu\text{mol/L}$, respectively) and in

mGFJ (0.6 ± 0.1 , 0.05 ± 0.02 , and 727 ± 14 $\mu\text{mol/L}$, respectively) were consistent with those measured initially (GFJ: 59 ± 2 , 0.71 ± 0.03 , and $1,012 \pm 24$ $\mu\text{mol/L}$; mGFJ: 0.3 ± 0.01 , 0.03 ± 0.01 , and 787 ± 69 $\mu\text{mol/L}$).^[5,13] The net loss of $\leq 3\%$ indicated negligible degradation over the >5-year storage period.

Table 1. Concentrations of Representative Furanocoumarins, Polymethoxyflavones, and Flavanones in Grapefruit Juice (GFJ) and Modified GFJ (mGFJ)

Constituent	Mean Concentration ($\mu\text{mol/L}$) (SD)	
	GFJ	mGFJ
Furanocoumarins		
DHB	38.8 (0.3)	0.13 (0.01)
Bergamottin	9.07 (0.04)	0.50 (0.08)
Polymethoxyflavones		
Nobiletin	0.37 (0.02)	0.02 (0.01)
Tangeretin	0.11 (0.01)	0.03 (0.01)
Flavanones		
Naringin	770 (5)	531 (8)
Narirutin	271 (4)	183 (5)
Hesperidin	24.6 (1.2)	14.7 (0.6)

DHB, 6',7'-dihydroxybergamottin.

Effects of Grapefruit Juice and Modified Grapefruit Juice Extracts on Estrone 3-Sulfate and Fexofenadine Uptake in OATP-Transfected Cells

Ethyl acetate extracts of GFJ and mGFJ were evaluated as inhibitors of OATP1A2- and OATP2B1-mediated uptake in COS-1 and HEK293T/17 cells using the probe substrate estrone 3-sulfate (E1S) to assess functional activity of the juices prior to clinical study conduct. Both juice extracts inhibited E1S uptake in COS-1 cells by at least 50% relative to vehicle (Figure 1A and B). Single-strength GFJ and mGFJ extracts inhibited OATP1A2-mediated uptake by 70% and 80%, respectively; double-strength extracts inhibited uptake by 84% and 50%, respectively (Figure 1A). Single-strength GFJ and mGFJ extracts inhibited OATP2B1-mediated uptake by 78% and 61%, respectively; double-strength extracts inhibited uptake by 52 and 74%, respectively (Figure 1B). Both juice extracts inhibited E1S uptake in HEK293T/17 cells by at least 45% relative to vehicle (Figure 1C and D). Single-strength GFJ and mGFJ extracts inhibited OATP1A2-mediated uptake by 90% and 62%, respectively; double-strength extracts inhibited uptake by 80% and 74%, respectively (Figure 1C). Single-strength GFJ and mGFJ extracts inhibited OATP2B1-mediated uptake by 74% and 59%, respectively; double-strength extracts inhibited uptake by 60% and 45%, respectively (Figure 1D). BSP, a non-specific OATP inhibitor, inhibited uptake by >80% in all cell systems.

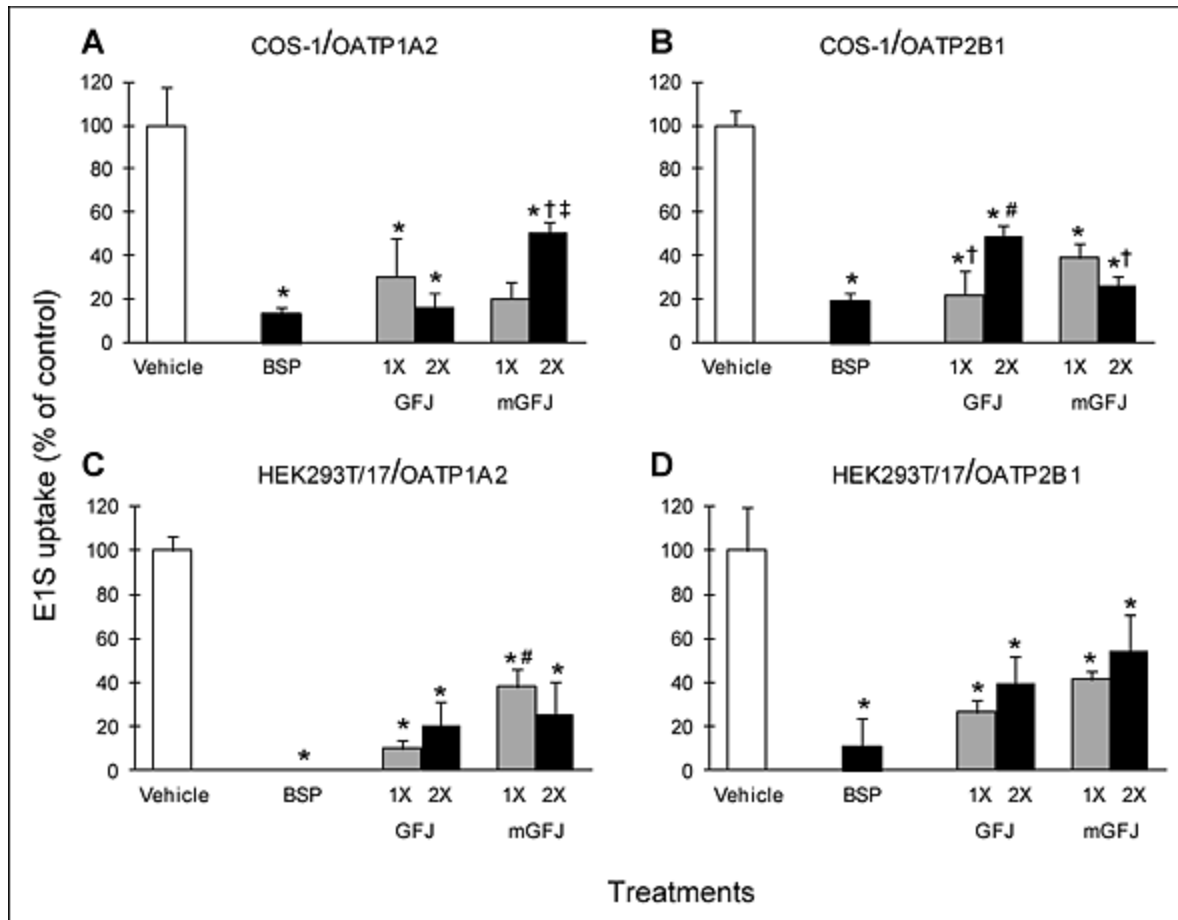


Figure 1. Inhibitory effects of grapefruit juice extracts (GFJ, mGFJ) on estrone 3-sulfate (E1S) uptake in COS-1 (A and B) and HEK293T/17 (C and D) cells overexpressing human OATP1A2 (A and C) or OATP2B1 (B and D). Cells were transiently transfected with human OATP1A2, OATP2B1, or mock (EYFP) plasmids. Cells were incubated for 3 minutes in pH 6.0 buffer at 37°C with 0.05 $\mu\text{mol/L}$ [^3H]E1S in the presence of vehicle (1% methanol) or GFJ and mGFJ extracts at single-strength (1 \times) or double-strength (2 \times). Bromosulphophthalein (BSP) (250 $\mu\text{mol/L}$) was used as a positive control inhibitor. E1S mean net uptake by OATP1A2 in the presence of vehicle was 17.5 ± 3.2 and 17.3 ± 1.1 pmol/mg protein/3 minutes, for COS-1 and HEK293T/17 cells, respectively. E1S mean net uptake by OATP2B1 in the presence of vehicle was 42.7 ± 3.0 and 17.7 ± 3.5 pmol/mg protein/3 minutes for COS-1 and HEK293T/17 cells, respectively. Bars and error bars denote means and SDs, respectively, of triplicate incubations. * $P < .05$ versus vehicle (two-way ANOVA followed by Tukey's test), # $P < .05$ versus GFJ 1 \times (two-way ANOVA followed by Tukey's test), † $P < .05$ versus GFJ 2 \times (two-way ANOVA followed by Tukey's test), ‡ $P < .05$ versus mGFJ 1 \times (two-way ANOVA followed by Tukey's test).

COS-1 cells transiently overexpressing OATP1A2 were employed initially to gain mechanistic insight into inhibition of fexofenadine uptake by GFJ and mGFJ. Due to high uptake activity in mock (EYFP)-transfected cells with the juice extracts and BSP (Figure 2A), OATP1A2 was expressed transiently in HEK293T/17 cells, in which uptake in mock-transfected cells was more consistent (Figure 2B). Relative to vehicle-treated OATP1A2-transfected cells, both single- and

double-strength GFJ extract inhibited fexofenadine uptake by ~27%. Both single- and double-strength mGFJ extract inhibited uptake by ~40%. Because BSP did not inhibit fexofenadine uptake in HEK293T/17 cells, VER was used as an alternate OATP1A2 inhibitor, inhibiting by ~50%.

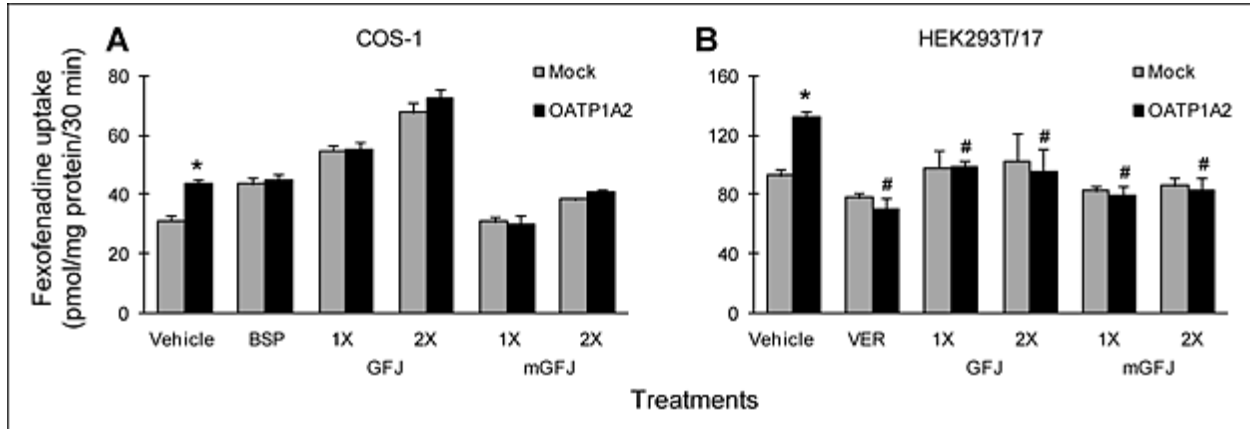


Figure 2. Inhibition of fexofenadine uptake by grapefruit juice extracts (GFJ, mGFJ) in OATP1A2-transfected cells. COS-1 (A) and HEK293T/17 (B) cells were transiently transfected with human OATP1A2 (black bars) or mock (EYFP) plasmids (gray bars). Cells were incubated for 30 minutes in pH 6.0 buffer at 37°C with 0.5 $\mu\text{mol/L}$ [^3H]fexofenadine in the presence of vehicle (1% methanol) or GFJ and mGFJ extracts at single-strength (1 \times) or double-strength (2 \times). Bromosulfophthalein (BSP) and verapamil (VER) (250 $\mu\text{mol/L}$) were used as positive control inhibitors. Fexofenadine mean net uptake by OATP1A2 in the absence of juice extracts was 12.8 ± 1.1 and 39.4 ± 4.8 pmol/mg protein/30 minutes, for COS-1 and HEK293T/17 cells, respectively. Bars and error bars denote means and SDs, respectively, of triplicate incubations. * $P < .05$ versus mock-transfected cells (Student's unpaired t -test). # $P < .05$ versus vehicle-treated OATP1A2-transfected cells (two-way ANOVA followed by Tukey's test).

OATP2B1-mediated uptake of fexofenadine was evaluated in transiently transfected COS-1 and stably transfected MDCKII cells (Figure 3). Due to the possibility of cell line-dependent expression/uptake, OATP2B1 was expressed in HEK293T/17 cells. Fexofenadine uptake was not observed with any cell line.

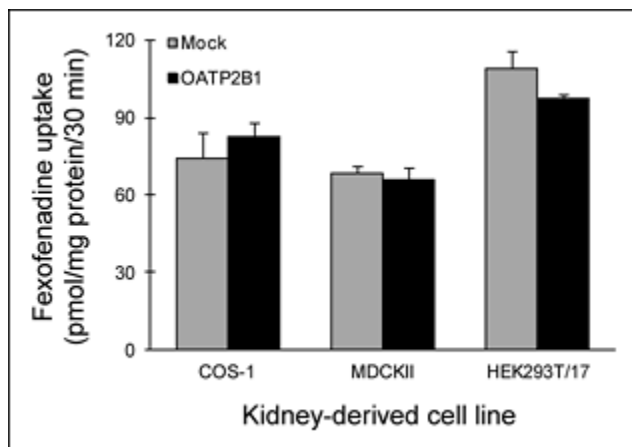


Figure 3. Fexofenadine uptake in OATP2B1-transfected cells. Transiently transfected COS-1 cells and HEK293T/17 cells and stably transfected MDCKII cells were incubated for 30 minutes in pH 6.0 buffer at 37°C with 0.5 $\mu\text{mol/L}$ [^3H]fexofenadine. Estrone 3-sulfate (E1S) was used as a positive control; fold-difference between control and transfected cells was 4, 5, and 20 for COS-1, MDCKII, and HEK293T/17 cells, respectively. Black bars denote OATP2B1-mediated uptake. Gray bars denote mock-transfected (COS-1, HEK293T/17) or parental cell uptake (MDCKII). Bars and error bars denote means and SDs, respectively, of triplicate incubations.

Effects of Grapefruit Juice and Modified Grapefruit Juice on Fexofenadine Pharmacokinetics in Healthy Volunteers

The effects of GFJ and mGFJ on fexofenadine pharmacokinetics were compared in 18 healthy participants. None of the participants withdrew from the study. Each treatment was well tolerated by all participants. No side effects were reported.

The geometric mean concentration–time profiles of fexofenadine in the presence of GFJ and mGFJ were nearly superimposable (Figure 4). The percentage of fexofenadine AUC extrapolated to infinite time ($\text{AUC}_{0-\infty}$) was $<10\%$ in all subjects and in all phases. Relative to water, GFJ and mGFJ decreased geometric mean $\text{AUC}_{0-\infty}$ by $\sim 25\%$ (Table 2). The geometric mean $\text{AUC}_{0-\infty}$ s were similar between GFJ and mGFJ (Table 2). Both juices increased geometric mean Cl/F by $\sim 33\%$ (Table 2). Relative to water, both juices decreased geometric mean C_{max} by $\sim 23\%$ (Table 2). The geometric mean terminal $t_{1/2}$ and median t_{max} did not differ between treatments (Table 2).

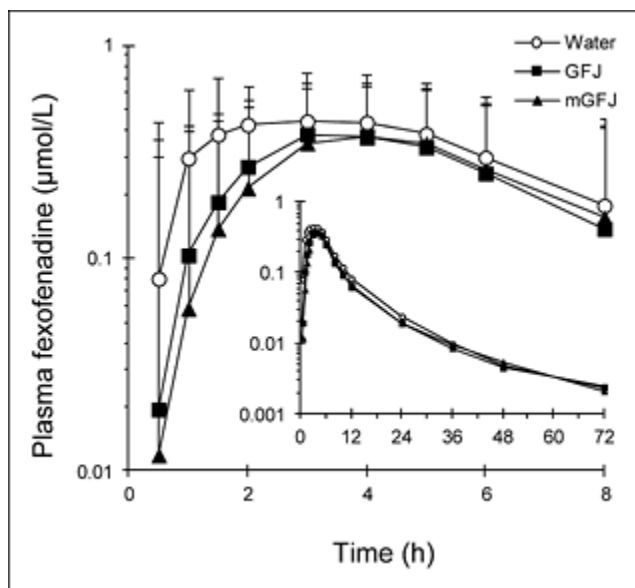


Figure 4. Geometric mean plasma fexofenadine concentration–time profile following co-administration with 240 mL of water, grapefruit juice (GFJ), or modified GFJ (mGFJ) for 18 healthy volunteers. Symbols and error bars denote geometric means and upper limits of the 90% confidence interval, respectively. Inset depicts the 0–72 hours profile. The 2-hour time point of the water phase represents the

geometric mean of 17 subjects. Open circles, closed squares, and closed triangles denote water, GFJ, and mGFJ, respectively.

Table 2. Pharmacokinetics of Fexofenadine (120 mg) in 18 Healthy Volunteers After Administration With 240 mL of Water, Grapefruit Juice (GFJ), or Modified GFJ (mGFJ)

Outcome	Geometric Mean (CV%)			Geometric Mean Ratio (90% CI)	
	Water	GFJ	mGFJ	GFJ/Water	GFJ/mGFJ
AUC _{0-∞} (μmol/L h)	4.22 (40.0)	3.22 ^a (33.5)	3.15 (28.6)	0.76 (0.3–1.4)	0.98 (0.4–1.5)
Cl/F (L/h)	52.8 (40.0)	69.3 ^b (33.5)	70.9 (29.6)	1.31 (0.70–1.92)	1.02 (0.45–1.60)
C _{max} (μmol/L)	0.57 (52.2)	0.45 ^c (43.7)	0.44 (32.4)	0.78 (0.17–1.39)	0.97 (0.35–1.60)
t _{1/2} (h)	11.9 (36.5)	10.3 ^d (37.6)	10.3 (29.7)	0.86 (0.28–1.45)	1.01 (0.43–1.58)
t _{max} (h) [median (range)]	3 (1–6)	3.5 ^e (1.5–5)	4 (2–6)		

AUC_{0-∞}, area the curve from time zero to infinity; Cl/F, apparent oral clearance; C_{max}, maximum concentration; t_{1/2}, terminal half-life; t_{max}, time to C_{max}.

Statistical comparisons for all outcomes except t_{max} were made between water and GFJ and between GFJ and mGFJ using a one-tailed paired Student's *t*-test with Bonferroni correction (*p* < 0.025).

a *P* = 0.008.

b *P* = 0.023.

c *P* = 0.011.

d *P* = 0.11.

e *P* = 0.135 (Wilcoxon signed-rank test).

Discussion

Since discovery of the fruit juice–OATP interaction, candidate inhibitors of OATP isoforms such as OATP1A2 and OATP2B1 have been proposed and evaluated for effects on intestinal and hepatic drug uptake.^[17-20] The GFJ and mGFJ tested in the current work were compared previously in two clinical studies involving the CYP3A substrate felodipine and the dual CYP3A/P-gp substrate cyclosporine. The felodipine study established furanocoumarins, in aggregate, as major inhibitors of enteric CYP3A.^[5] The cyclosporine study further substantiated furanocoumarins as major inhibitors of enteric CYP3A, and likely P-gp; in addition, polymethoxyflavones were ruled out as inhibitors of enteric P-gp.^[13] Based on these observations, this unique GFJ-mGFJ combination enabled both in vitro and clinical evaluation of the collective impact of furanocoumarins and polymethoxyflavones on the absorption of a growing class of drugs whose enteric uptake depends on OATPs. Because no “clean” OATP substrates suitable for human use have been identified, coupled with prior knowledge of the

GFJ–fexofenadine interaction,^[6-10] fexofenadine was selected as a third prototypic probe substrate to test with this GFJ and mGFJ.

Before clinical testing, both the original GFJ and mGFJ were evaluated for OATP inhibitory activity using OATP1A2- and OATP2B1-overexpressing cell systems and the probe substrate E1S. Compared to vehicle, both juice extracts inhibited both OATP isoforms by >50% in COS-1 and HEK293T/17 cells. Inhibition of E1S uptake by both extracts also was observed in stably transfected MDCKII–OATP2B1 cells (data not shown) and was consistent with previous observations with stably transfected HEK293 cells and dilutions of whole GFJ.^[12] The similar extents of OATP inhibition by the two extracts in HEK293T/17 cells predicted that systemic fexofenadine exposure in the clinical study would be comparable between juice treatments.

As anticipated, relative to water, GFJ decreased the geometric mean systemic exposure (AUC, C_{max}) to fexofenadine. This observation, coupled with the lack of an effect on geometric mean terminal half-life, was consistent with inhibition of uptake in the intestine by GFJ. The decrease in AUC (~25%) was lower than that reported in previous GFJ–fexofenadine studies (31–67%),^[6-10] which could be attributed to the highly variable concentrations of bioactive ingredients among commercial brands and batches of GFJ.^[21, 22] The modest decrease in AUC reflects considerable interindividual variability in magnitude of effect, which ranged from –68 to +57% among the 18 subjects. This variability could be attributed in part to polymorphisms in the genes that encode for OATP (*SLCO*), as well as P-gp (*MDR1*); however, genotyping for relevant polymorphisms was beyond the scope of this study.

As predicted by the enteric OATP-transfected cells using E1S as the probe substrate, mGFJ decreased geometric mean systemic fexofenadine exposure to a similar extent as GFJ. The nearly identical effects by both juices on fexofenadine pharmacokinetics indicated furanocoumarins and polymethoxyflavones are not major mediators of the GFJ–fexofenadine interaction. Elimination of furanocoumarins as major *in vivo* inhibitors of enteric OATPs is consistent with the single clinical study involving 12 healthy volunteers administered fexofenadine (120 mg) and an aqueous suspension (300 mL) of a particulate fraction of GFJ, which contained mostly furanocoumarins (measurements not provided) and a relatively trivial concentration (34 $\mu\text{mol/L}$) of the flavanone naringin.^[10] Fexofenadine AUC in the presence of the particulate fraction was similar to that in the presence of water. Naringin also was tested in the same clinical study^[10] at the same concentration as that in whole GFJ (~1,200 $\mu\text{mol/L}$); the single constituent explained only half of the reduction in fexofenadine exposure caused by GFJ, indicating other constituents, possibly other flavanones (e.g., narirutin, hesperidin), contribute to the interaction.

As aforementioned, fexofenadine is not a clean OATP substrate. Numerous investigators have shown that fexofenadine also is a P-gp substrate.^[23-27] The diluted extracts of the clinically administered juices were tested as inhibitors of fexofenadine uptake in various OATP1A2- or OATP2B-transfected kidney-derived cell lines. Initial optimization studies with COS-1 cells demonstrated that fexofenadine was a weak substrate for OATP1A2. Low fexofenadine uptake

by OATP1A2, coupled with high background activity, rendered difficulty in ascertaining the effects of the juice extracts. However, unlike COS-1 cells, the juice extracts did not significantly alter fexofenadine uptake in mock-transfected HEK293T/17 cells. Due to higher fexofenadine uptake activity in OATP1A2-transfected HEK293T/17 cells, comparable inhibition by both juice extracts was demonstrated. In addition to P-gp and OATP, apical and basolateral efflux mediated by multidrug resistant protein (MRP) 2 and MRP3, respectively, have been shown to contribute to fexofenadine transport in vitro.^[28] Studies with transiently transfected COS-1 and HEK293T/17 cells, as well as stably transfected MDCKII cells, demonstrated that fexofenadine is not a substrate for OATP2B1. Conflicting in vitro data have been reported regarding OATP2B1-mediated transport of fexofenadine. Fexofenadine uptake in transiently transfected HeLa and stably transfected HEK293 cells either was not evident or was weak.^[9, 29, 30] However, an independently generated MDCKII–OATP2B1 cell line showed an ~3-fold difference in fexofenadine uptake compared to mock-transfected cells.^[28] Fexofenadine has been shown to be a substrate of OATP2B1 in a *Xenopus laevis* oocyte system.^[31] The discrepancy between in vitro experiments with fexofenadine may be attributed to multiple binding sites on OATP2B1 with different affinities, but to date, substrate-dependent binding has been demonstrated only with transfected oocytes.^[32]

In summary, current in vitro and clinical observations collectively indicate that furanocoumarins and polymethoxyflavones are not major mediators of the GFJ–fexofenadine interaction. Flavanones are likely candidate inhibitors of enteric OATPs in vivo. In vitro and clinical studies accounting for natural product ingredient composition are essential for appropriate evaluation, translation, and optimal pharmacotherapeutic management of GFJ- and other natural product–drug interactions. A bioactivity-guided fractionation approach similar to that used to identify intestinal CYP3A inhibitors in cranberry^[33] and hepatic OATP inhibitors in *Rollinia emarginata*^[34] can be used to identify specific causative ingredients. Identification of such OATP inhibitors would enable evaluation and prediction of the interaction liability of GFJ and other foods containing the same compounds with other existing and potential OATP substrates, including some fluoroquinolones, beta-blockers, and statins.^[35] Finally, confirmation of causative mediators may impact food manufacturing practices, leading to advances designed to minimize interference with drugs, including removal of compounds via chemical processing^[5] or plant gene engineering.^[36]

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Declaration of Conflicting Interests

The authors have no disclosures and declare no personal or financial conflicts of interest.

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