PHARMACOKINETICS AND DRUG DISPOSITION

Seville orange juice-felodipine interaction: Comparison with dilute grapefruit juice and involvement of furocoumarins

Objective: **Our objective was to determine whether Seville orange juice produces a grapefruit juice–like interaction with felodipine and whether bergamottin, 6′,7′-dihydroxybergamottin, or other furocoumarins are involved.**

Methods: **In a randomized three-way crossover design, 10 volunteers received a felodipine 10-mg extended-release tablet with 240 mL of Seville orange juice, dilute grapefruit juice (that contained equivalent total molar concentrations of bergamottin plus 6′,7′-dihydroxybergamottin), or common orange juice (negative control). The pharmacokinetics of felodipine and its dehydrofelodipine metabolite were determined. Juice concentrations of furocoumarins were measured. CYP3A4 inhibitory activity of newly identified furocoumarins was assessed.**

Results: **The felodipine area under the plasma concentration-time curve was increased by 76% and 93% after Seville orange juice and grapefruit juice ingestion, respectively, compared with common orange juice. The effects of Seville orange juice and grapefruit juice were similar in that the felodipine maximum concentration was augmented while the terminal elimination half-life was unchanged and the dehydrofelodipine area under the plasma concentration time-curve was increased, but the dehydrofelodipine-felodipine area under the plasma concentration-time curve ratio was reduced. Bergamottin and 6′,7′-dihydroxybergamottin concentrations were 5 and 36 μmol/L, respectively, in Seville orange juice and were 16 and 23 μmol/L, respectively, in dilute grapefruit juice. A newly identified furocoumarin, bergapten, was detected only in Seville orange juice (31 μmol/L), and it was found to be a mechanism-based inhibitor of recombinant CYP3A4. Relative to the control, 6′,7′-dihydroxybergamottin (10 μmol/L) inhibited CYP3A4 activity in cultured intestinal epithelial cells by 93%, whereas bergapten (10 μmol/L) inhibited the activity by only 34%.**

Conclusions: **Seville orange juice and grapefruit juice interact with felodipine by a common mechanism, which is probably inactivation of intestinal CYP3A4. Bergamottin and 6′,7′-dihydroxybergamottin may be "marker substances" in foods for this interaction. The lack of interaction between Seville orange juice and cyclosporine (INN, ciclosporin) suggests that grapefruit juice may also inhibit intestinal P-glycoprotein, whereas Seville orange juice may selectively "knock out" intestinal CYP3A4. (Clin Pharmacol Ther 2001;69:14-23.)**

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Since the first report that showed that grapefruit juice increases the oral availability of felodipine,[1] the list of grapefruit juice–drug interactions has been expanded to more than 20 medications from a diverse range of therapeutic categories including cardiovascular, antihistaminic, gastrointestinal, antiinfective, antilipidic, central nervous system, and immunosuppressive agents.[2] Each drug that interacts with grapefruit juice is a substrate for cytochrome P450 (CYP)3A4, the major CYP isoform expressed in adult human small intestine[3] and liver.[4] Because grapefruit juice does not alter the elimination half-life of drugs administered orally or intravenously, it is believed that the "grapefruit juice effect" is primarily due to inhibition of intestinal rather than hepatic CYP3A4.[5]

Grapefruit juice consumption has been shown to cause a prompt decrease in intestinal CYP3A4 immunoreactive protein in humans; this is consistent with mechanism-based inactivation of the enzyme followed by rapid intracellular degradation.[5,6] Moreover, certain furocoumarins (psoralens) found in grapefruit juice decreased CYP3A4 immunoreactive protein in cultured intestinal epithelial (Caco-2) cells [6] or inhibited CYP3A4 catalytic activity, either reversibly or by mechanism-based inactivation, in human intestinal and liver microsomes.[6-10] Such furocoumarins include bergamottin,[8] 6′,7′-dihydroxybergamottin,[6,7] and at least four others.[6,9,10] Because bergamottin and 6′,7′dihydroxybergamottin appear to be the most abundant furocoumarins in grapefruit juice,[6-10] these two substances may largely account for the grapefruit juice effect. Clinical testing is necessary, however, to identify the inhibitors of intestinal CYP3A4 in grapefruit juice.[11]

For the assessment of the effects of bergamottin and 6′,7′-dihydroxybergamottin in vivo, it would be preferable to administer these substances as pure compounds directly to humans. Unfortunately, the compounds are not yet approved for human intake. An alternative approach is to find another food that contains bergamottin and 6′,7′-dihydroxybergamottin in appropriate concentrations and then test administer this food with an established drug probe to determine whether it produces an interaction similar to that observed with grapefruit juice.

We have determined that juice prepared from Seville (or sour) oranges contains bergamottin as well as 6′,7′-dihydroxybergamottin, as previously reported.[12] In addition, Seville orange juice ingestion in 2 healthy volunteers resulted in a dramatic decrease in intestinal CYP3A4 content,[12] which is consistent with results reported for grapefruit juice.[5]

We conducted an interaction study with Seville orange juice and felodipine, the most extensively studied probe for grapefruit juice–drug interactions.[2] The results were compared with those from grapefruit juice diluted to contain equivalent total molar concentrations of bergamottin plus 6′,7′-dihydroxybergamottin (positive control) and with those from common (or sweet) orange juice, which neither interacts with felodipine[1] nor contains these furocoumarins[10,11] (negative control). Compared with common orange juice, both Seville orange juice and grapefruit juice similarly enhanced the oral availability of felodipine and altered the disposition of its CYP3A4-mediated metabolite, dehydrofelodipine. Seville orange juice was also found to contain another furocoumarin, bergapten (5-methoxypsoralen), which inhibited CYP3A4 but was less potent than 6′,7′-dihydroxybergamottin. Because our previous report had shown that Seville orange juice did not augment plasma cyclosporine (INN, ciclosporin) concentrations compared with grapefruit juice,[12] this suggests that grapefruit juice may also clinically inhibit intestinal P-glycoprotein–mediated drug efflux and that Seville orange juice may selectively "knock out" intestinal CYP3A4.

MATERIALS AND METHODS

Materials

Frozen concentrated grapefruit juice and common orange juice were obtained from a local market and diluted to regular strength. Seville orange juice was prepared by squeezing the fresh fruit, which was purchased from Russo Co (Watertown, Mass) and then sweetening it with sugar to make it more palatable. The juices were stored at –20°C until needed for the study. Before administration, the reconstituted grapefruit juice was diluted 1.3:1 with water; this resulted in equivalent total molar concentrations of bergamottin and 6′,7′-dihydroxybergamottin as in the Seville orange juice. Saquinavir and midazolam were gifts from Roche Laboratories (Nutley, NJ). Indinavir was a gift from Merck and Co (West Point, Pa). Ketoconazole and troleandomycin were purchased from Sigma Chemical Co (St Louis, Mo). Bergamottin was purchased from Indofine Chemical Co (Somerville, NJ). 6′,7′-Dihydroxybergamottin was generously supplied by Dr Tony Montanari and Mr Bill Widmer (Department of Citrus, Lake Alfred, Fla). Bergapten (5-methoxypsoralen) and all-trans retinol were purchased from Aldrich Chemical Co (Milwaukee, Wis). Human complementary deoxyribonucleic acid (cDNA)–expressed CYP3A4 was purchased from Gentest (Woburn, Mass). J' sphere ODS-M80 HPLC columns were obtained from YMC Co, Ltd (Wilmington, NC). All other chemicals were reagent or tissue culture grade where appropriate.

Human subject study

This study was approved by the University of Michigan Institutional Review Board. Ten healthy nonsmoking volunteers (5 men and 5 women) who ranged in age from 21 to 32 years enrolled in the study after providing written informed consent. Prestudy evaluations showed that all of the subjects had normal findings on physical examination and routine laboratory tests that included hematology, serum chemistry, and urinalysis. None of the subjects was taking medications, including those available over the counter.

A three-way randomized crossover study design was used in which each subject received a single oral dose of a felodipine 10-mg extended-release tablet (Plendil; Merck & Co, West Point, Pa) with 240 mL of Seville orange juice, dilute grapefruit juice, or common orange juice. Peripheral venous blood (7 mL) was collected just before felodipine and juice administration and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours thereafter. Plasma was separated from blood cells by centrifugation and stored at –20°C until analysis. The subjects fasted overnight and were provided lunch and dinner, devoid of fruits and vegetables, at 4 and 8 hours after dosing, respectively. All subjects refrained from consuming alcohol or caffeine-containing beverages beginning 2 weeks before the study and during the course of the study. The interval between each juice treatment was at least 1 week.

Analysis of plasma for felodipine and dehydrofelodipine

Plasma concentrations of felodipine and its primary metabolite, dehydrofelodipine, were quantified by a previously published method with minor modifications.[13] In brief, 200 µL of plasma was extracted with 200 µL of toluene that contained the internal standard, H165/04 (AB Haessle, Gothenburg, Sweden), by means of gentle oscillation of the mixture overnight. After centrifugation, 1 µL of toluene extract was introduced by autoinjector and splitless injection into a dual tapered deactivated glass insert (Hewlett-Packard Canada, Toronto, Ontario) to prevent chemical oxidation of felodipine in the injector port. Chromatography was accomplished with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a ^{63}Ni electron capture detector and a 25 m × 0.32 mm internal diameter fused silica capillary column coated with the stationary phase methyl silicone 0.52 µm (HP-1; Hewlett-Packard). After purge for 1 minute, the oven temperature of 90°C was increased 30°C per minute to 180°C, then 5°C per minute to 260°C for 3 minutes, and then 30°C per minute to a final temperature of 280°C for 5 minutes. The injector port temperature was maintained at 260°C, and the detector temperature was maintained at 300°C. The carrier gas was ultrapure helium (column inlet pressure of 100 kPa), and the makeup gas was ultrapure nitrogen (60 mL/min). The retention times for felodipine, dehydrofelodipine, and internal standard were 20.1, 14.5, and 21.7 minutes, respectively. Each plasma sample was analyzed in duplicate. The interday coefficients of variation in plasma felodipine and dehydrofelodipine at 5 nmol/L were 3.5% (n = 8) and 9.6% (n = 8), respectively. The limit of detection for both felodipine and dehydrofelodipine was 0.5 nmol/L.

Pharmacokinetic analysis

Felodipine and dehydrofelodipine disposition were assessed with the use of noncompartmental methods. The terminal elimination rate constant (λ_z) was determined with linear regression of at least the last three data points from the natural logarithm of the plasma concentration-time curve. The terminal elimination half-life ($t_{½}$) was calculated as $\ln 2/\lambda_z$. The zero to 24-hour area under the plasma concentration-time curve was calculated by the linear trapezoidal method. Plasma peak concentration (C_{max}) and the time to reach C_{max} (t_{max}) were obtained directly from the concentration-time profiles.

Statistical analyses

All statistical analyses were performed with the use of StatView (v5.0.1, SAS Institute, Inc, Cary, NC). Comparisons among the three treatment groups were initially made with ANOVA with repeated measures. For those analyses for which *P* was less than .05, comparisons between Seville orange juice or grapefruit juice and common orange juice were made with the paired *t* test and a Bonferroni-corrected level of significance (*P* = .025).

Analysis of furocoumarin concentrations in citrus juices

Bergamottin. An aliquot (1.0 mL) of Seville orange juice or grapefruit juice used in the study was extracted thrice with dichloromethane (1.0 mL) by shaking for 1 hour. After centrifugation, the dichloromethane extracts were combined, the internal standard (all-trans retinol) was added, and the solution was evaporated to dryness under a gentle stream of nitrogen in an ice bath. (We found that drying on ice was essential for recovery of bergamottin, presumably because of evaporation. Failure to use this procedure probably accounts for the lower bergamottin concentrations obtained in our earlier study.[5]) The residue was dissolved in HPLC mobile phase (1.0 mL) that consisted of acetonitrile:water (80:20 vol/vol) and triethylamine (400 µL/L) adjusted to a pH of 8.0 with 0.01 N sodium hydroxide. An

aliquot of the solution (100 μL) was filtered (0.45 μm), and a sample (25 μL) was injected onto a 150 × 3.2 mm Prodigy C_{18}, 5 μm column (Phenomenex, Torrance, Calif) at a flow rate of 0.7 mL/min. Ultraviolet absorbance was measured at 310 nm. The retention times for bergamottin and internal standard were 5.2 and 8.4 min, respectively. The ultraviolet absorbance profiles (270-350 nm) of the chromatographic peak from Seville orange juice and grapefruit juice were identical to that of pure bergamottin standard. Samples were analyzed in triplicate. The absolute recovery of bergamottin standard freshly prepared in common orange juice, which does not contain this substance, was 91% ± 2%. The standard curve was linear over the tested range (0-25 μmol/L) and had an intraday coefficient of variation of 1.7% at 10 μmol/L (n = 4).

6′,7′-Dihydroxybergamottin and bergapten (5-methoxypsoralen). An aliquot (1.0 mL) of Seville orange juice or grapefruit juice was extracted once with dichloromethane (1.0 mL) by shaking for 1 hour. After centrifugation, a portion (100 μL) of the dichloromethane extract was evaporated to dryness under nitrogen in an ice bath. The residue was dissolved in HPLC mobile phase (100 μL) that consisted of acetonitrile: water (35:65 vol/vol) and triethylamine (400 μL/L) adjusted to a pH of 3.0 with phosphoric acid. The solution was filtered (0.45 μm) and a sample (25 μL) was injected onto a 150 × 3.2 mm Prodigy C_{18}, 5 μm column at a flow rate of 0.7 mL/min. Ultraviolet absorbance was measured at 310 nm. The retention times for 6′,7′-dihydroxybergamottin and bergapten were 12.3 and 9.5 minutes, respectively. The ultraviolet absorbance profiles (270-350 mm) for the chromatographic peaks were the same as those for standard 6′,7′-dihydroxybergamottin and bergapten. The assignment of the relevant peak to bergapten was further confirmed by mass spectrometric analysis. Samples were analyzed in triplicate. The standard curves for 6′,7′-dihydroxybergamottin and bergapten were prepared in common orange juice, which does not contain these substances, and coincided with those made directly in HPLC mobile phase; this demonstrated complete recovery of these furocoumarins by the extraction procedure. The standard curves were linear over the tested range (0-50 μmol/L). The intraday coefficients of variation for 6′,7′-dihydroxybergamottin and bergapten at 25 μmol/L were 5.8% (n = 5) and 2.8% (n = 5), respectively.

Inhibition of cDNA-expressed human CYP3A4 by bergapten

Saquinavir was chosen as the substrate for these studies because of its very high turnover, a prerequisite for the mechanism-based inhibition study. For the reversible inhibition study, 22 μL of cDNA-expressed human CYP3A4 (200 pmol/mL) was preincubated with 10 μL of freshly prepared NADPH-generating system (yielding final concentrations of 1 mmol/L $NADP^+$, 10 mmol/L of glucose-6-phosphate, and 1 U/mL of glucose-6-phosphate dehydrogenase) and 950 μL of phosphate buffer (0.1 mol/L of potassium phosphate, 1 mmol/L of ethylenediaminetetraacetic acid, 5 mmol/L magnesium chloride, pH 7.4) at 37°C. After 3 minutes, 10 μL of 0.1, 1, or 10 mol/L bergapten was added, followed by 10 μL of 0.5 mmol/L saquinavir. Bergapten and saquinavir were both dissolved in acetonitrile:water (1:1 vol/vol). After an additional 15 minutes, the reaction was stopped by the addition of 1 mL of ice-cold acetonitrile and immediate placement of the sample on ice.

For the mechanism-based inhibition study, 25 μL of cDNA-expressed CYP3A4 (2 nmol/mL) was preincubated with 1.2 μL of NADPH-generating system and 93 μL of phosphate buffer. After 3 minutes, 10 μL of bergapten was added to achieve final concentrations of 0, 0.1, 1, 10, or 100 μmol/L. After 0, 15, and 30 minutes, 10 μL of reaction mixture was removed and added to 990 μL of a solution composed of 10 μL of 0.5 mmol/L of saquinavir, 10 μL of NADPH-generating system, and 970 μL of phosphate buffer. The reaction was terminated after 15 minutes as described previously.

CYP3A4 activity was assessed by measurement of saquinavir metabolite (M-7) formation as previously described.[14] In brief, internal standard (4 mL of 0.1 μmol/L indinavir in acetonitrile) was added to the incubate and evaporated to dryness with a Savant SpeedVac system (Holbrook, NY). The remaining residue was dissolved in 200 μL of acetonitrile:water (1:1 vol/vol). Two milliliters of acetonitrile was then added. The precipitate was removed by centrifugation, and the samples were dried again. The final residue was dissolved in 15 μL of acetonitrile:water (1:1 vol/vol), and 12 μL was injected onto a Prism HPLC column (Keystone Scientific Inc, Bellafonte, Pa) connected to a Hewlett-Packard 1100 series HPLC coupled to a Finnigan ion trap LCQ mass spectrometer (Thermoquest, San Jose, Calif). M-7 was eluted with water and 0.1% (vol/vol) formic acid in acetonitrile. The initial eluant was held at 90% water for 1 minute. The 0.1% formic acid in acetonitrile was increased linearly to 65% over 27 minutes and then to 90% over 5 minutes. Water was then increased to initial conditions over 5 minutes. The mass spectrometer was operated in the positive electrospray ionization mode with the following conditions: electrospray voltage, 5.0 kV; sheath gas, 60 (arbitrary units); auxiliary gas, 20 (arbitrary units); capillary tempera-

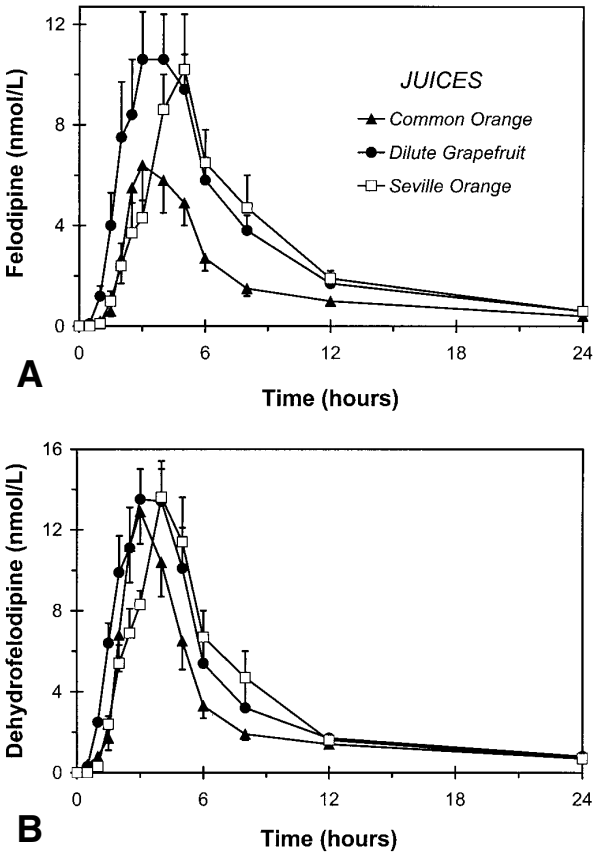

Fig 1. Mean plasma concentration-time profiles for felodipine **(A)** and dehydrofelodipine **(B)** in 10 healthy subjects after administration of a felodipine 10-mg extended-release tablet with 240 mL of Seville orange juice, dilute grapefruit juice, or orange juice. *Error bars* denote standard errors.

ture, 225°C; capillary voltage, 25 V; tube offset voltage, 36 V; and scan range, 600 to 700 mass-to-charge ration. Internal standard (indinavir) and M-7 were extracted from the total ion current with the use of mass-to-charge ratios of 614.4 and 687.4, respectively.

Inhibition of CYP3A4 by 6′,7′-dihydroxybergamottin and bergapten in Caco-2 cell monolayers

Midazolam was chosen as the substrate for these studies because (1) we were unable to adequately measure felodipine metabolites in the incubation medium, (2) unlike saquinavir, midazolam appears not to be a substrate for P-glycoprotein,[15] which is known to be highly expressed in Caco-2 cells, and (3) midazolam has been well-characterized as a CYP3A probe in our cell system.[16-18] The Caco-2 cell subclone P27.7 was cultured on laminin-coated inserts and treated with the CYP3A4 inducing agent, 1α,25-dihydroxyvitamin D_3, for 2 weeks, beginning at confluence as previously described.[16] Incubation medium (serum-free differentiation medium) was freshly prepared and kept at 37°C. All compounds were dissolved as 1000-fold concentrated solutions in either absolute ethanol (6′,7′-dihydroxybergamottin and ketoconazole) or dimethyl sulfoxide (midazolam, bergapten, and troleandomycin). The incubation medium was spiked with compound(s) or vehicle (0.1% ethanol and 0.2% dimethyl sulfoxide) just before its addition (1.5 mL) to the apical chamber of duplicate cultures; this was followed by an equal volume of plain incubation medium to the basolateral chamber. Cell cultures were first preincubated with either 10 μmol/L of 6′,7′-dihydroxybergamottin or bergapten (10 μmol/L) for 30 minutes and then incubated simultaneously with 6′,7′-dihydroxybergamottin or bergapten and midazolam (3 μmol/L) for 4 hours. As a positive control, separate cultures were incubated similarly with the selective mechanism-based CYP3A4 inhibitor troleandomycin (30 μmol/L). Another set of cultures was also incubated simultaneously with the reversible CYP3A4 inhibitor ketoconazole (1 or 10 μmol/L) and midazolam for 2 hours. Medium collected from the apical and basolateral compartments of each culture was analyzed for 1′-hydroxymidazolam with gas chromatography-mass spectrometry as previously described.[16]

RESULTS

Effect of Seville orange juice and dilute grapefruit juice on oral felodipine disposition

The effect of each juice on mean plasma concentrations and pharmacokinetics of felodipine and its primary metabolite, dehydrofelodipine, are shown in Fig 1 and Table I. On average, the area under the plasma concentration-time curve (AUC) and C_{max} of felodipine were increased by 76% and 61%, respectively, with Seville orange juice and by 93% and 88%, respectively, with grapefruit juice compared with common orange juice. The differences in AUC and C_{max} observed between Seville orange juice and grapefruit juice were not significant. The t_{max} and terminal $t_{½}$ of felodipine were not different among juice treatments. Compared with common orange juice, the AUC of dehydrofelodipine was increased by 30% with Seville orange juice and by 35% with grapefruit juice. C_{max} and the terminal $t_{½}$ of dehydrofelodipine were not significantly different among the juices. The AUC ratios of dehydrofelodipine to felodipine were significantly lower with both Seville orange juice and grapefruit juice compared with common orange juice.

Table I. Pharmacokinetics of felodipine and its dehydrofelodipine metabolite after a single dose administration of a felodipine 10-mg extended-release tablet with 240 mL of Seville orange juice, dilute grapefruit juice, or common orange juice.

	Seville orange juice	*Dilute grapefruit juice*	*Orange juice*
Felodipine			
$AUC_{0\text{-}24}$ (nmol/L · h)	68.1 ± 8.7*	74.7 ± 8.8*	38.6 ± 5.5
C_{max} (nmol/L)	11.9 ± 1.9*	13.9 ± 1.7*	7.4 ± 1.4
t_{max} (h)	4.4 ± 0.5	3.4 ± 0.4	3.4 ± 0.3
Terminal $t_{½}$ (h)	5.9 ± 0.5	7.7 ± 1.3	8.8 ± 1.5
Dehydrofelodipine			
$AUC_{0\text{-}24}$ (nmol/L · h)	81.0 ± 9.5*	84.4 ± 8.3*	62.4 ± 6.5
C_{max} (nmol/L)	16.7 ± 1.6	17.0 ± 1.5	14.4 ± 1.5
t_{max} (h)	3.9 ± 0.3*	2.8 ± 0.3	2.8 ± 0.2
Terminal $t_{½}$ (h)	10.9 ± 3.6	11.5 ± 2.6	11.3 ± 2.1
Dehydrofelodipine to felodipine			
$AUC_{0\text{-}24}$	1.3 ± 0.1*	1.2 ± 0.1*	1.8 ± 0.2

$AUC_{0\text{-}24}$, AUC from 0 to 24 hours.
Data are presented as mean values ± standard error of the mean. Comparisons are between Seville orange juice or dilute grapefruit juice and common orange juice.
*$P < .025$.

Furocoumarin concentrations in Seville orange juice and dilute grapefruit juice

The bergamottin and 6′,7′-dihydroxybergamottin concentrations were 5 μmol/L and 36 μmol/L, respectively, in Seville orange juice and were 16 μmol/L and 23 μmol/L, respectively, in dilute grapefruit juice. The bergapten concentration in Seville orange juice was 31 μmol/L. Bergapten was not detected in dilute grapefruit juice (<1 μmol/L).

Inhibition and inactivation of cDNA-expressed human CYP3A4 by bergapten

Preliminary kinetic studies showed that metabolism of saquinavir to its major metabolite, M-7, was reversibly inhibited by bergapten with a 50% inhibitory concentration that ranged from 10 to 100 μmol/L (data not shown). Subsequently, bergapten was shown to produce time- and concentration-dependent inhibition of the enzyme (Fig 2).

Inhibition of CYP3A4 activity in Caco-2 cells by 6′,7′-dihydroxybergamottin and bergapten

6′,7′-Dihydroxybergamottin (10 μmol/L) inhibited midazolam 1′-hydroxylation by 93% compared with control, whereas the same concentration of bergapten inhibited this activity by only 34% (Fig 3). Comparatively, the CYP3A4 inhibitors troleandomycin (30 μmol/L) and ketoconazole (1 and 10 μmol/L) inhibited midazolam 1′-hydroxylation by 60% and at least 75%, respectively. Mean control activities for mechanism-based inhibitor- and ketoconazole-treated cultures were 95 and 107 pmol per hour per culture, respectively.

DISCUSSION

Felodipine is generally completely absorbed from the gastrointestinal tract after oral administration.[19] However, it suffers from high presystemic (first-pass) metabolism sequentially in the gut and then the liver; this results in a low absolute oral bioavailability, which averages 15%.[19,20] Felodipine is biotransformed to a single inactive primary metabolite, dehydrofelodipine,[21] and then to a major secondary metabolite (M3) by CYP3A4.[22,23]

Compared with common orange juice, Seville orange juice and grapefruit juice increased the AUCs of felodipine and dehydrofelodipine but decreased the dehydrofelodipine-felodipine AUC ratio. The decrease in the metabolite-parent AUC ratio is consistent with inhibition of the primary metabolic pathway.[2] The absolute increase in the AUC of dehydrofelodipine suggested that a subsequent metabolic step was inhibited; this has been supported for grapefruit juice by measurements that have shown that M3 was reduced.[2,23] Seville orange juice and grapefruit juice augmented the C_{max} but did not change the terminal $t_{½}$ of felodipine. Thus Seville orange juice and grapefruit juice appear to act by a common mechanism of action, which is probably inhibition of CYP3A4-mediated first-pass metabolism in the small intestine. These findings show that another food substance can cause grapefruit juice-like drug interactions. Because Seville oranges are used in the preparation of certain confectioneries, such as marmalades, it is possible that confectioneries made from this citrus fruit may also produce a drug interaction. However, it is not clear at this time whether sufficient concentrations of CYP3A4 inhibitors are present in those confectioneries to produce a drug interaction.

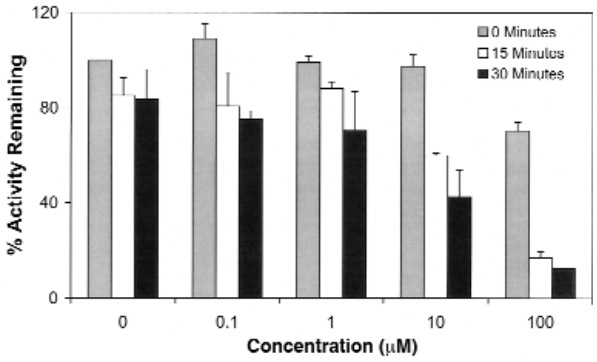

Fig 2. Mechanism-based inhibition of cDNA-expressed human CYP3A4 by bergapten. Expressed CYP3A4 was preincubated with an NADPH-generating system and with 0, 0.1, 1, 10, or 100 μmol/L of bergapten for 0, 15, and 30 minutes. Ten μL of the reaction mixture were removed and diluted 1:100 with phosphate buffer that contained 5 μmol/L of saquinavir and NADPH-generating system. Relative CYP3A4 activity (saquinavir oxidation) was determined as the percentage of control (based on peak areas). Each *bar* represents the mean value of 2 or 3 incubations. *Error bars* denote standard deviations.

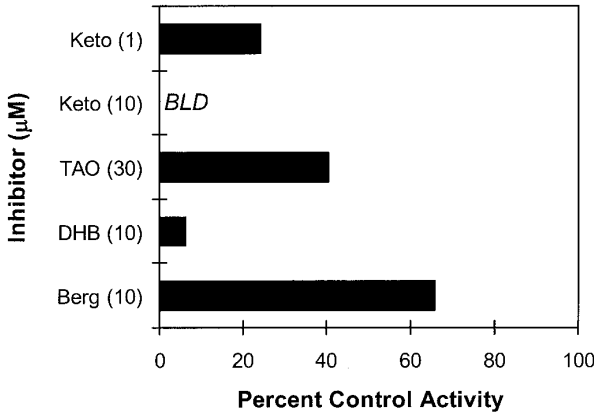

Fig 3. Effects of the CYP3A4 inhibitors ketoconazole (keto) and troleandomycin (TAO) and the furocoumarins 6′,7-dihydroxybergamottin (DHB) and bergapten (berg) on midazolam 1′-hydroxylation in Caco-2 cells expressing CYP3A4. Percent control activity was calculated as the total amount (apical + basolateral) of 1′-hydroxymidazolam formed in inhibitor or furocoumarin-treated cultures divided by total 1′-hydroxymidazolam formed in vehicle-treated cultures. Each *bar* represents the mean of duplicate cultures. Mean control activity for TAO, DHB, and bergapten-treated cultures was 95 pmol/h, and that for ketoconazole-treated cultures was 107 pmol/h. BLD, below limit of detection.

The Seville (or sour) orange is considered to be an ancient fruit whose existence was documented before the birth of Christ.[24] In contrast, the grapefruit is relatively new; it was first found growing in the West Indies in the mid-1700s. The grapefruit is believed to have evolved from the pummelo, either as a spontaneous mutation or an inadvertent hybrid with the common orange.[24,25] This is supported by the observation that juice from the pummelo contains several of the same furocoumarins as grapefruit juice and potently inhibits CYP3A4 activity in vitro.[10] Additional citrus fruits, including the Seville orange, may also have evolved from the pummelo.[25] This raises the possibility that the CYP3A4 inhibitors in the pummelo, grapefruit, and Seville orange are in other citrus fruits and that this type of food-drug interaction could be more common than previously thought. Indeed, juice from the Sweetie fruit has recently been shown to contain these furocoumarins and to inhibit CYP3A4 activity in vitro.[10]

In a grapefruit juice–felodipine interaction study in which small bowel biopsy specimens were obtained from healthy subjects, grapefruit juice effectively decreased enterocyte CYP3A4 protein levels.[5] Hepatic CYP3A4 activity, as measured by the intravenous erythromycin breath test, was not altered. The decrease in enterocyte CYP3A4 protein by grapefruit juice was not accompanied by a change in CYP3A4 messenger ribonucleic acid content; this suggests that the effect of grapefruit juice was acceleration of the degradation of enterocyte CYP3A4 protein rather than decreasing of its synthesis. It was concluded that substances in grapefruit juice are probably metabolized by CYP3A4 to reactive intermediates that inactivate the enzyme,[5,6] a process termed *mechanism-based inactivation*. The inactivated and structurally modified CYP3A4 presumably undergoes rapid proteolysis within the enterocyte.

Because the mechanism-based inhibitors bergamottin and 6′,7′-dihydroxybergamottin were present in both Seville orange juice and grapefruit juice, the results of this study support our hypothesis that these chemicals are involved in the clinical interaction and that they might be used as marker substances to predict this type of interaction with other foods. This possibility is supported by other findings. In a previous clinical investigation, grapefruit juice was first separated by high-speed centrifugation and ultrafiltration into two fractions (supernatant and particulate) that contained different concentrations of these two substances.[11] Each fraction was then administered with felodipine to test for an interaction. The supernatant fraction, which con-

tained no measurable bergamottin (<1 μmol/L) but contained 20 μmol/L of 6′,7′-dihydroxybergamottin, significantly augmented the AUC of felodipine compared with water. However, the effect was less pronounced compared with whole grapefruit juice. The rediluted particulate fraction, which contained 30 μmol/L of bergamottin and 6 μmol/l of 6′,7′-dihydroxybergamottin, also increased the AUC of felodipine to a greater extent than the supernatant fraction. This is consistent with the conclusion that both bergamottin and 6′,7′-dihydroxybergamottin are present in sufficient concentrations in grapefruit juice to produce in vivo inhibition of intestinal CYP3A4 and an interaction with felodipine. However, it now seems likely that additional furocoumarins contribute significantly to the effects of the whole juices.[6,9,10] For example, it has recently been reported that grapefruit juice and Seville orange juice contain the furocoumarins termed GF-I-1, GF-I-4, GF-I-5, and GF-I-6.[10] Each of these can produce both reversible and mechanism-based inhibition of CYP3A4 in human liver microsomes at concentrations approximately the same as those found in the juices.[10] In addition, GF-I-1 (also called FC726), has been shown to potently inhibit CYP3A4 and cause loss of CYP3A4 immunoreactive protein in cultured cells.[6] All of these furocoumarins are highly lipophilic and are concentrated primarily in the particulate fraction of grapefruit juice.[10] In this study and our previous studies,[26] these newly characterized furocoumarins were not measured, and thus their contribution to the observed effects in vivo is unknown.

We determined that Seville orange juice also contained another furocoumarin, bergapten. Bergapten produced mechanism-based inhibition of recombinant CYP3A4 and inhibited CYP3A4-mediated midazolam 1′-hydroxylation in intestinal cell culture at concentrations less than those measured in Seville orange juice. However, the in vitro potency of bergapten was about one third of that observed with 6′,7′-dihydroxybergamottin. Thus the contribution of bergapten to the in vivo interaction observed between Seville orange juice and felodipine is uncertain. Nevertheless, bergapten may be another furocoumarin capable of producing a clinical drug interaction.

In a recent study that was similar in design to the current investigation, grapefruit juice enhanced the oral availability of cyclosporine, whereas Seville orange juice did not.[12] This lack of effect by Seville orange juice occurred despite a marked decrease in enterocyte CYP3A4 content that was measured in 2 subjects. Therefore these results further support the conclusion that the extent of metabolism of cyclosporine by intestinal CYP3A4 is generally small.[27] It also follows that inhibition of intestinal CYP3A4 cannot be the main mechanism whereby grapefruit juice enhances the oral availability of cyclosporine.

P-glycoprotein is an efflux pump located in the apical membrane (brush border) of enterocytes.[28] P-glycoprotein functions to pump many xenobiotics, including cyclosporine, from the interior of the enterocyte back into the gut lumen.[29] Felodipine, however, does not appear to be a substrate for this pump.[30] The effect of grapefruit juice on P-glycoprotein mediated drug efflux has been investigated in two studies in which polarized human intestinal cells were used.[30,31] In one study, grapefruit juice inhibited P-glycoprotein–mediated efflux,[31] whereas in the other it appeared to activate P-glycoprotein–mediated efflux.[30] The authors of the latter work,[30] however, have recently stated that their findings may have been the result of equipment-generated artifacts (Leslie Z. Benet, PhD, personal communication, September 2000). Therefore the results of our study and in vitro findings support the concept that grapefruit juice may inhibit both intestinal-mediated CYP3A4 metabolism and P-glycoprotein efflux of drugs. Seville orange juice, on the other hand, may more selectively knock out enteric CYP3A4.

Because bergamottin and 6′,7′-dihydroxybergamottin were measured in both Seville orange juice and grapefruit juice, and because bergapten was present in only Seville orange juice, these three substances are unlikely to be clinically important inhibitors of intestinal P-glycoprotein. Naringin concentrations in the Seville orange juice and grapefruit juice used in the current study were measured according to a previously published method[11] and were found to be 1644 μmol/L and 439 μmol/L, respectively, which also eliminates this substance (a flavonoid) as an important inhibitor of P-glycoprotein in vivo. These conclusions are further supported by in vitro findings that show that bergamottin, 6′,7′-dihydroxybergamottin,[12,32] and naringin[32] did not inhibit P-glycoprotein–mediated efflux in cultured cells.

In summary, Seville orange juice and grapefruit juice produced a pharmacokinetic interaction with the drug probe felodipine, most probably as a result of inactivation of intestinal CYP3A4. Both bergamottin and 6′,7′-dihydroxybergamottin appear to be clinically important inhibitors in these juices, although other furocoumarins, including the newly identified substance bergapten, may be involved. Grapefruit juice may also inhibit intestinal P-glycoprotein–mediated drug efflux activity, although the responsible substances in grapefruit juice appear to be different from those identified as inactivating CYP3A4. Because Seville orange juice

may selectively knock out intestinal CYP3A4 activity, it might be used to estimate the relative contribution of the intestine to the overall first-pass metabolism of a CYP3A4 substrate.

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