Bergamottin contribution to the grapefruit juice–felodipine interaction and disposition in humans

Objectives: **Our objectives were to evaluate the contribution of bergamottin to the grapefruit juice–felodipine interaction and to characterize bergamottin disposition.**

Methods: **In this study 250 mL grapefruit juice; 2-, 6-, or 12-mg capsules of bergamottin plus water; or water was administered with 5 mg extended-release felodipine to 11 volunteers in a partially randomized, 5-way crossover study. Plasma concentrations of felodipine, its primary metabolite (dehydrofelodipine), bergamottin, and 6′,7′-dihydroxybergamottin were determined.**

Results: **Grapefruit juice (containing 1.7 mg bergamottin) increased peak plasma concentration (C_{max}) and area under the plasma concentration–time curve (AUC) of felodipine by 89% ($P < .025$) and 54% ($P < .025$), respectively, compared with water. With 2 mg bergamottin, felodipine C_{max} increased by 33% ($P < .05$). The increase by bergamottin was markedly variable among individuals (range, −33% to 125%). With 6 mg bergamottin, felodipine C_{max} was enhanced by 35% ($P < .025$), and with 12 mg bergamottin, felodipine C_{max} increased by 40% ($P < .05$) and AUC increased by 37% ($P < .05$) compared with water. Bergamottin measured in plasma after administration of 6 and 12 mg produced C_{max} values of 2.1 and 5.9 ng/mL, respectively, and times to reach C_{max} of 0.8 and 1.1 hours, respectively. The bergamottin metabolite 6′,7′-dihydroxybergamottin was detected in plasma of some subjects after bergamottin administration.**

Conclusions: **Bergamottin enhanced the oral bioavailability of felodipine and may cause a clinically relevant drug interaction in susceptible individuals. Grapefruit juice–drug interactions likely also involve other furanocoumarins, possibly acting in combination by additive or synergistic mechanisms. Bergamottin has systemic availability and is metabolized in vivo to 6′,7′-dihydroxybergamottin. (Clin Pharmacol Ther 2004; 76:607-17.)**

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Grapefruit juice was initially shown to increase the oral bioavailability of medications from a wide range of therapeutic categories, including the antihypertensive dihydropyridine calcium antagonist felodipine.[1] Felodipine is normally completely absorbed from the gastrointestinal tract after oral administration but has a low absolute bioavailability (15%) as a result of extensive presystemic metabolism mediated by cytochrome P450 (CYP) 3A4.[2] Grapefruit juice coadministration resulted in a several-fold increase in peak plasma concentration

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Supported in part by grants from the Medical Research Council of South Africa (02M03) and Canadian Institute of Health Research (MOP 67013).

Presented in part at the Fifty-second Annual Meeting of the American Society for Mass Spectrometry, Nashville, Tenn, May 23-27, 2004.

Received for publication June 7, 2004; accepted Aug 25, 2004.

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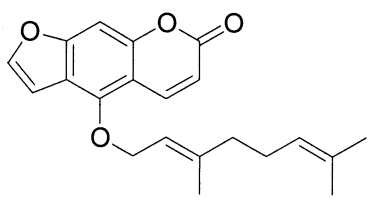
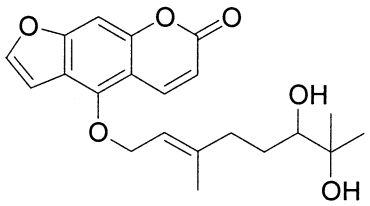
0009-9236/$30.00

doi:10.1016/j.clpt.2004.08.019



Bergamottin



6',7'-Dihydroxybergamottin

Fig 1. Chemical structures of bergamottin and 6′,7′-dihydroxybergamottin.

(C_{max}) and the area under the plasma concentration–time curve (AUC) of felodipine and its primary metabolite, dehydrofelodipine, and a decrease in the dehydrofelodipine/felodipine AUC ratio.[3,4] This was attributed to inhibition of both the primary and secondary steps in the metabolism of felodipine. Grapefruit juice at normal volume did not change the terminal half-life ($t_{½}$) or intravenous pharmacokinetics of drugs.[1,5-7] Therefore this pharmacokinetic interaction is thought to be due primarily to grapefruit juice–mediated inhibition of intestinal CYP3A4 activity without apparent inhibition of hepatic CYP3A4 activity. Grapefruit juice inhibition of CYP3A4 in vivo appears to involve irreversible inactivation of CYP3A4, as evidenced by down-regulation of intestinal CYP3A4 protein content without alteration of intestinal messenger ribonucleic acid levels.[3,8] This posttranslational mechanism most likely resulted from mechanism-based inactivation of intestinal CYP3A4, ultimately resulting in protein degradation.[9] Evidence further suggests that grapefruit juice might also inhibit P-glycoprotein, although clinical support is still controversial.[10-15] The grapefruit juice interaction has subsequently been shown to be relevant for a number of medications including terfenadine, cisapride, halofantrine, lovastatin, simvastatin, amiodarone, buspirone, tacrolimus, and cyclosporine (INN, ciclosporin).[3,16]

Initial studies investigating the effect of flavonoids as in vivo CYP3A4 inhibitors failed to show significant activity in humans.[17-19] More recent efforts have focused on furanocoumarins. Bergamottin (Fig 1) is present in grapefruit juice in concentrations ranging from 2 to 30 μmol/L,[12,20,21] depending on the source of the juice. These concentrations approach or exceed those for half-maximal enzyme inactivation (K_I) determined by use of expressed CYP3A4 (7.7 μmol/L)[22] or human liver microsomes (40 μmol/L).[23] The 50% inhibitory concentration for nifedipine oxidation determined with recombinant protein (5.4 μmol/L)[24] is also lower than in human liver microsomes (22 μmol/L, testosterone 6β-hydroxylation).[21] The more hydrophilic metabolite, 6′,7′-dihydroxybergamottin, is often present in grapefruit juice in similar concentrations (0.8 to 58 μmol/L),[21,25] but initial investigations indicated that it is a less potent in vitro mechanism-based inactivator of CYP3A4 (K_I, 59 μmol/L) than bergamottin.[25] However, recent reports by different groups indicate that 6′,7′-dihydroxybergamottin is a more potent mechanism-based inactivator or inhibitor of CYP3A4.[23,26] Interestingly, 50% inhibitory concentration values determined for inhibition of CYP3A4 activity vary by 2 orders of magnitude and range between 0.3 to 26 μmol/L.[24-28] Clinically, 6′,7′-dihydroxybergamottin administered as the aqueous fraction of grapefruit juice increased the oral bioavailability of felodipine. The magnitude of interaction indicated that 6′,7′-dihydroxybergamottin was unlikely to be the primary active constituent.[29] However, a recent study in a smaller population suggested a larger contribution of 6′,7′-dihydroxybergamottin than previously reported.[30]

Bergamottin is both a competitive inhibitor and a mechanism-based inactivator of CYP3A4 in human liver microsomes and recombinant complementary deoxyribonucleic acid–expressed protein.[21-23] In addition, bergamottin has been shown to be a substrate inhibitor of P-glycoprotein in whole cell–based assays.[31] Because mechanism-based inactivation can result in protein degradation in vivo and bergamottin is one of the major furanocoumarins in the juice, bergamottin might be a principal constituent responsible for the interaction in humans.[22,32] Administration of a particulate portion of grapefruit juice (containing high concentrations of bergamottin) enhanced bioavailability of felodipine more than the supernatant portion (containing no detectable bergamottin and high concentrations of 6′,7′-dihydroxybergamottin).[29] However, dilute lime juice containing the same concentration of bergamottin produced much less interaction than did whole grapefruit juice.[33] Bergamottin coadministration increased the bioavailability of diazepam in beagle dogs by 54-fold and the bioavailability of cyclosporine and nifedipine in rats by 3.4-fold and 1.5-fold,

respectively.[34-36] However, relative exposure to bergamottin is not known, and doses administered were typically larger than those encountered by humans after normal consumption of grapefruit juice. Thus the relevance of bergamottin in the clinical interaction of grapefruit juice in humans is currently uncertain.

Determining the role of bergamottin might ideally involve administering this furanocoumarin as a pure substance. The purpose of this study was to assess the effect of 3 escalating doses of pure bergamottin, administered as an ethanolic solution in gelatin capsules, on the oral pharmacokinetics of felodipine in humans. Results showed that bergamottin at a dose similar to that in grapefruit juice augmented the oral bioavailability of felodipine but the interaction was much less than that produced by the juice.

METHODS

Materials

Grapefruit juice concentrate (Cedar Lite; Bromor Foods, Cape Town, South Africa) was obtained from a local market. Bergamottin was purchased from Indofine Chemical Co (Somerville, NJ) and was of the highest purity (100%) commercially available. Purity was confirmed by HPLC (>99.8%) with ultraviolet (UV) detection (310 nm)[21] and liquid chromatography–tandem mass spectrometry (LC-MS/MS) analyses essentially as later described. Trioxsalen (INN, trioxysalen) and bergapten were purchased from Sigma-Aldrich (St Louis, Mo), and 6′,7′-dihydroxybergamottin was supplied by Ultrafine Chemicals (Manchester, England). All other materials were reagent-grade and obtained from commercial sources.

Analysis of bergamottin concentration in grapefruit juice

Bergamottin concentration in grapefruit juice concentrate (Cedar Lite; Bromor Foods) was analyzed essentially according to a previously published method.[15] Grapefruit juice was diluted with water, and an aliquot (1 mL) was extracted twice with an equal volume of ethyl acetate and once with dichloromethane by shaking for 45 minutes. Extracts were combined after centrifugation, dried under a gentle stream of nitrogen in an ice bath, and dissolved in 200 μL mobile phase (65% acetonitrile) containing 100 nmol of trioxsalen as internal standard. The grapefruit juice components were separated by HPLC (Agilent Hewlett-Packard 1100; Chemetrix Ltd, Midrand, South Africa) equipped with a diode array detector. An aliquot (20 μL) was injected onto a C18 column (Luna, 5 μm, 4.6 × 250 mm; Phenomenex, Torrance, Calif) at a flow rate of 1 mL/min, and elution was performed with 100-mmol/L acetic acid (A) and acetonitrile (B) by a gradient of 40% B for 5 minutes and then 40% to 70% B within 25 minutes and held for 5 minutes before returning to initial conditions. UV absorbance was measured at 310 nm and quantified according to a standard curve generated between 1 and 100 μmol/L. The standard curve was linear over the range tested ($r^2 > 0.999$) and had a coefficient of variation of 4.2% at 1 μmol/L (n = 3). The lower limit of quantitation for bergamottin was 0.5 μmol/L, and the interday coefficient of variation was 5.9% at 20 μmol/L. Bergamottin and trioxsalen eluted at 31.9 and 14.2 minutes, respectively, and bergamottin recovery from spiked orange juice (not containing bergamottin) was 97% ± 6% (n = 3). The coefficient of variation for extracted bergamottin was 8.2% at 20 μmol/L. Analysis for assay specificity was performed by coinjection and indicated no split peaks. In addition, the UV absorption profile (200 to 350 nm) of the assayed chromatographic peak from the ethyl acetate/dichloromethane extract was identical to that of pure bergamottin. Concentrations of other furanocoumarins in the juice were not quantified because of the lack of commercially available standards at the time of analysis.

Human pharmacokinetic study

Subjects. Twelve white male volunteers (age range, 21-24 years) gave written informed consent before entering the study as approved by the Ethics Committee for Human Research of North-West University (Potchefstroom, South Africa) (02M03). As a result of the limited availability of Good Manufacturing Practice bergamottin and individual regulations on food substances, future studies may not be permitted in some countries. The clinical study was performed at the Drug Bioavailability Centrum, Department of Pharmacology, North-West University, and was conducted in accordance with the Declaration of Helsinki. The subjects were ascertained to be healthy by medical history, physical examination, routine hematologic testing, and blood and urine chemistry studies. All subjects had a normal 12-lead electrocardiogram and tested negative for human immunodeficiency virus 1 or 2 antibody, hepatitis B antigen, or hepatitis C antibody at screening. None of the subjects was receiving any medication on a long-term basis, and all were purported to be nonsmokers.

Bergamottin capsules. Bergamottin dosing solutions were prepared in absolute ethanol to yield stock solutions of 4, 12, or 24 mg/mL directly before administration on each experimental day. Soft gelatin cap-

sules were filled with 500 μL of the respective stock solutions for oral administration of 2, 6, or 12 mg bergamottin. Bergamottin was used without further physicochemical characterization. However, the purity of bergamottin in dosing solutions was confirmed by liquid chromatography–diode array detector analyses by use of an HPLC system equipped with a diode array detector for UV detection, as well as subsequent LC-MS/MS analyses by use of the same chromatographic separation conditions described for the analysis of bergamottin in grapefruit juice. UV spectra were recorded over the range of 200 to 360 nm. As a result, only 1 peak was detected in our liquid chromatography–diode array detector analysis (>99.8% at λ_{max} = 310 nm, where λ_{max} is wavelength of maximum absorption) that was confirmed to be pure bergamottin on the basis of the subsequent mass spectrometric analysis results showing characteristic fingerprints of bergamottin. Mass spectra were recorded over the range of mass-to-charge ratio (*m/z*) 100 to 1000 for full scan analysis, and protonated molecule of bergamottin ($[M+H]^+$ = *m/z* 339) was detected as the base peak. Characteristic product ions of *m/z* 203, 175, and 137 were detected in product ion analysis that were consistent with previous findings.[22] No other peaks or ions possibly related to the synthesis materials (bergaptol or geranyl bromide) were detected.

Protocol. Subjects received racemic felodipine in a 5-mg extended-release formulation (Plendil; AstraZeneca, Sunninghill, South Africa) with (1) 250 mL grapefruit juice (Cedar Lite; Bromor Foods) as positive control or (2) water (negative control) in a randomized, 2-way crossover manner followed by 250 mL water with (3) 2 mg, (4) 6 mg, or (5) 12 mg bergamottin (gelatin capsule containing 500 μL ethanol) in a single-dose, randomized, 3-way crossover manner. The 2-mg, 6-mg, or 12-mg bergamottin dose administered would be equivalent to that encountered after consumption of approximately 1, 3.5, or 7 glasses (250 mL) of Cedar Lite grapefruit juice, respectively. The same lot number of grapefruit juice was used throughout the study. Peripheral venous blood (10 mL) was sampled just before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 24 hours after dosing for the purpose of measuring plasma felodipine, dehydrofelodipine, bergamottin, or 6′,7′-dihydroxybergamottin concentrations. Plasma samples were split for felodipine and bergamottin analyses and stored at −80°C after immediate centrifugation. Plasma was shipped under dry ice, and samples were stored at −80°C before analyses as described below. Subjects consumed standardized meals at 5 hours after dosing (1 PM) and 8 hours after dosing (4 PM). Consumption of beverages that contained caffeine was not allowed during testing, and water was allowed starting at 3 hours after dosing. Subjects were not permitted to consume alcohol for at least 48 hours and fasted for at least 10 hours before testing. They were instructed not to consume grapefruit juice products, other than the one used in the study, for 2 weeks before and during the period of investigation. Compliance to this criterion was determined by measuring predose plasma bergamottin concentrations in all subjects before each treatment phase. Subjects were prohibited from taking prescription drugs, over-the-counter medications, and herbal supplements within 30 days before and during the study. The washout interval between study days was 1 week. Patients were under the constant supervision of a licensed physician and registered nurse for the duration of the study. Adverse events were recorded by questionnaire, and volunteers were encouraged to immediately report any change in well-being.

Plasma analysis for felodipine and dehydrofelodipine. Plasma concentrations of felodipine and dehydrofelodipine were quantified by modification of a previously published method.[37] Plasma samples were shipped under dry ice to the Department of Medicine, London Health Sciences Centre, London, Ontario, Canada, and stored at −80°C until analysis. In brief, plasma (500 μL) was extracted with toluene (500 μL) containing the internal standard (H165/04; AB Haessle, Gotenburg, Sweden) by gentle oscillation of the mixture overnight. After centrifugation, a sample of the toluene extract (1 μL) was introduced by splitless injection into a dual-tapered deactivated glass insert to prevent chemical oxidation of felodipine in the injector port. Chromatography was performed with a Hewlett-Packard 5890 Series II Gas Chromatograph equipped with a nickel 63 electron capture detector and a fused silica capillary column (internal diameter, 0.32 mm × 25 m) coated with a stationary phase of methyl silicone (0.52 μm) (HP-1; Hewlett-Packard Canada Ltd, Mississauga, Ontario, Canada). After a purge for 1 minute, the initial oven temperature of 90°C was increased at 30°C/min to 180°C, at 5°C/min to 260°C for 3 minutes, and then at 30°C/min to a final temperature of 280°C for 5 minutes. The injector port and detector temperatures were maintained at 260°C and 300°C, respectively. The carrier gas was ultrapure helium (column inlet pressure, 100 kPa), and the makeup gas was ultrapure nitrogen (60 mL/min). The retention times of felodipine, dehydrofelodipine, and internal standard were 20.1, 14.5, and 21.7 minutes, respectively. The interday coefficients of variation for plasma felodipine and dehydrofelodipine concentration were 3.5% and

9.6%, respectively, at 5 nmol/L. The limit of quantitation was less than 0.5 nmol/L for each substance.

Plasma analysis for bergamottin and 6′,7′-dihydroxybergamottin. Plasma concentrations of bergamottin and 6′,7′-dihydroxybergamottin were quantified by LC-MS/MS. Plasma samples were shipped to the Department of Pharmacokinetics, Dynamics, and Metabolism, Pfizer Global Research and Development, Ann Arbor, Mich, and kept at −80°C until analysis. Plasma samples obtained before dosing and at 0.5, 1, 1.5, 2, 2.5, and 3 hours after dosing were analyzed, because initial experiments indicated no detectable bergamottin or 6′,7′-dihydroxybergamottin plasma concentrations at later times (4 to 24 hours).

Stock solutions of bergamottin, 6′,7′-dihydroxybergamottin, and bergapten (internal standard) were prepared as 5 mg/mL in dimethylsulfoxide and then diluted to 10 μg/mL with mobile phase. Stock solutions were then serially diluted by use of blank plasma as necessary. The linear regression of the standard curves for bergamottin and 6′,7′-dihydroxybergamottin showed $r^2 = 0.997$ and 0.999, respectively, over the range of 1 to 200 ng/mL.

For sample analysis, a 100-μL aliquot of each plasma sample was mixed with 250 μL of 50 ng/mL bergapten (internal standard) in acetonitrile and centrifuged at 4000 rpm at 4°C for 5 minutes. Supernatants were collected, evaporated to dryness, and reconstituted in 50 μL of mobile phase (50% acetonitrile/0.1% formic acid) for analysis. Aliquots (5 μL) of plasma extracts were injected directly onto a MetaChem Polaris C_{18} 50 × 2.0–mm, 5-μm column (ANSYS Technologies Inc, Torrance, Calif). Elution was performed with a step gradient of 50:50 (vol/vol) acetonitrile/0.1% formic acid (A) and 95:5 (vol/vol) acetonitrile/0.1% formic acid (B) at 4% B initial conditions, with a step to 50% B at 1.0 minute and a step to 96% B at 1.2 minutes, and held before returning to initial conditions at 4 minutes. The column was maintained at room temperature, and the flow rate was 0.25 mL/min, with a total run time of 4 minutes. There was a 2-minute re-equilibrium period with the initial solvent mixture between analyses. Bergamottin, 6′,7′-dihydroxybergamottin, and bergapten eluted at approximately 2.6, 1.4, and 1.6 minutes, respectively.

Positive ion electrospray tandem mass spectra were recorded by use of an Applied Biosystems/MDS-SCIEX model API 3000 triple-quadrupole mass spectrometer (Concord, Ontario, Canada) equipped with Applied Biosystems / MDS-SCIEX Analyst (version 1.2) operating software. The ion-spray voltage was set to 4500 V, and the probe temperature was set at 450°C. Nitrogen was used as the collision gas, and the nebulizer, curtain, and collision gases were set to 6, 10, and 5, respectively. Multiple reaction monitoring transitions of *m/z* 339.1 → 203.1, *m/z* 373.1 → 203.2, and *m/z* 217.2 → 202.1 were used during quantitative analysis of bergamottin, 6′,7′-dihydroxybergamottin, and bergapten, respectively, with a dwell time of 300 ms for each transition.

Data analysis. One volunteer (subject 11) was found to have a measurable concentration of bergamottin in the predose plasma sample during the water control period, and all data from this individual were excluded from analysis for this violation in protocol. Plasma felodipine and dehydrofelodipine concentrations were analyzed by a noncompartmental method. The terminal elimination rate constant (k_e) was determined by log-linear regression of the final data points (at least 3). The apparent elimination half-life ($t_{½}$) was calculated as $0.693/k_e$. The area under the plasma concentration–time curve (AUC) was calculated by the linear trapezoidal method. Maximal plasma drug concentration (C_{max}) and the time to reach C_{max} (t_{max}) for felodipine, dehydrofelodipine, bergamottin, and 6′,7′-dihydroxybergamottin were obtained directly from the experimental data.

Statistical comparisons among control treatments and bergamottin treatments were initially done by 3-way ANOVA for repeated measures performed with SAS for Windows software (version 8.02; SAS Institute Inc, Cary, NC). The analysis model included sequence, subject nested within sequence, and drug formulation as factors. No sequence (ie, carryover) effects were observed for any pharmacokinetic parameter. For those ANOVA analyses of treatment effect with $P < .05$, post hoc a priori comparisons were performed between water and the 4 treatments with the paired *t* test, with $P < .05$ being considered to indicate statistical significance. Results are presented as mean ± SD in tables and mean ± SEM in figures.

RESULTS

Bergamottin concentration in grapefruit juice

Grapefruit juice (Cedar Lite; Bromor Foods) contained 20.1 ± 0.2 μmol/L bergamottin, or approximately 1.7 mg/250 mL.

Human pharmacokinetic study

Effect of grapefruit juice and bergamottin. Grapefruit juice augmented plasma concentrations, C_{max}, and AUC of felodipine compared with water (Fig 2 and Table I). Felodipine C_{max} and AUC from 0 to 12 hours ($AUC_{0\text{-}12}$) were increased by 89% (range,

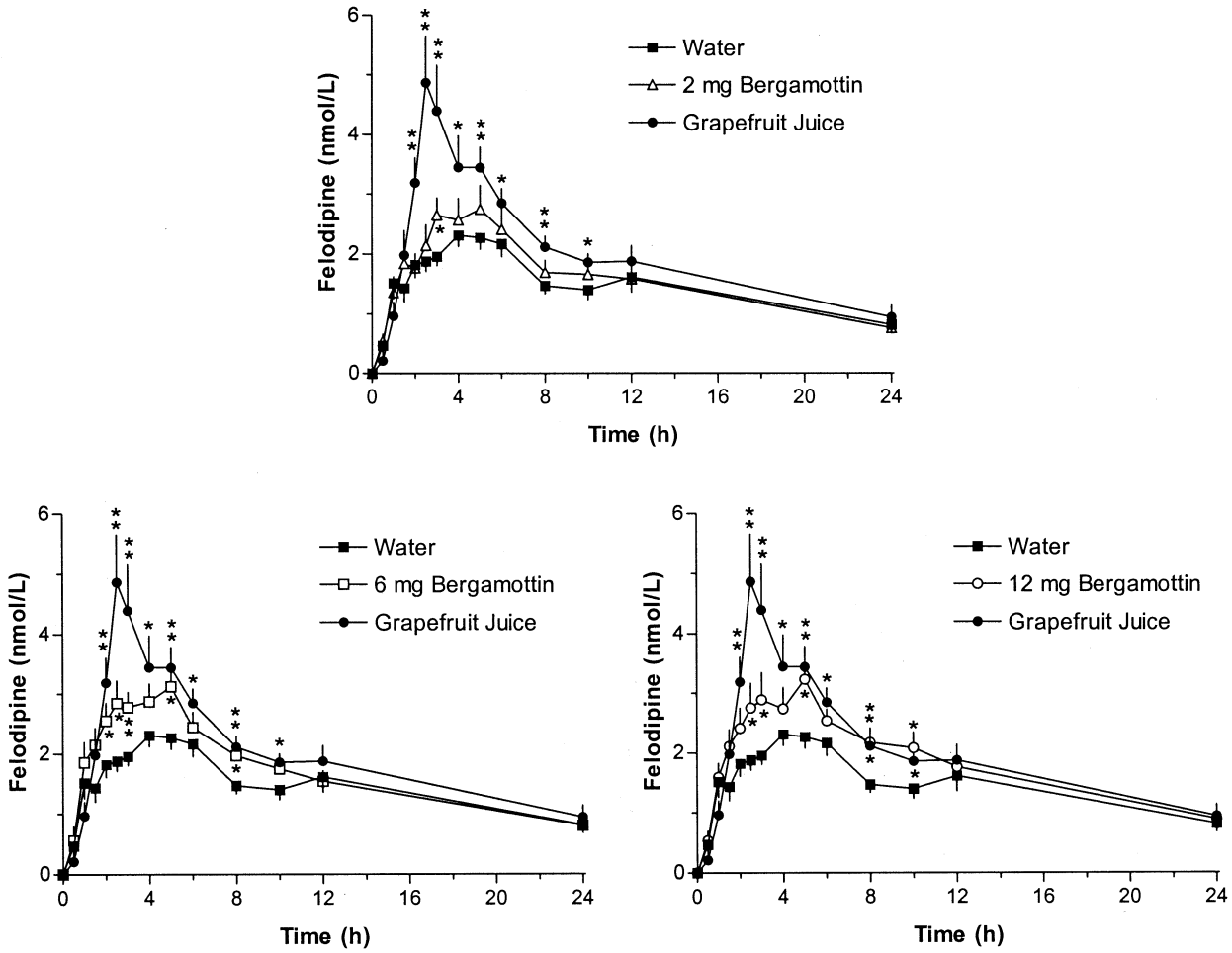


Fig 2. Mean plasma concentration–time profiles for felodipine with 250 mL grapefruit juice (*solid circles*); 2 mg (*open triangles*), 6 mg (*open squares*), or 12 mg bergamottin (*open circles*); or 250 mL water (*solid squares*) in 11 healthy subjects. *Error bars* denote SE. Comparisons were made at each measurement time between the 4 treatments and water: *1 asterisk*, $P < .05$; *2 asterisks*, $P < .01$.

−27% to 224%; $P < .025$) and 54% (range, −24% to 139%; $P < .025$), respectively, compared with water. Plasma dehydrofelodipine concentrations, AUC, and C_{max} values were increased. Dehydrofelodipine/felodipine AUC ratio (2.5 ± 0.2 versus 2.1 ± 0.2) and felodipine or dehydrofelodipine t_{max} or $t_{½}$ were not altered compared with those with water.

Bergamottin, 2 mg, increased felodipine C_{max} by 33% (range, −33% to 125%; $P < .05$) compared with water. Bergamottin, 6 mg, increased felodipine C_{max} by 35% (range, −30% to 86%; $P < .025$). Bergamottin, 12 mg, increased felodipine C_{max} by 40% (range, −9% to 120%; $P < .05$) and $AUC_{0\text{-}12}$ by 37% (range, −8% to 124%; $P < .05$) and dehydrofelodipine $AUC_{0\text{-}12}$ by 41% (range, −27% ± 103%; $P < .025$). Bergamottin administration did not affect dehydrofelodipine/felodipine AUC ratio or felodipine or dehydrofelodipine t_{max} or $t_{½}$.

The extent of the change in C_{max} and change in $AUC_{0\text{-}12}$ of felodipine with grapefruit juice ($r = 0.87$, $P < .001$) or 2 mg ($r = 0.75$, $P = .007$), 6 mg ($r = 0.72$, $P = .012$), or 12 mg bergamottin ($r = 0.77$, $P = .006$) was highly correlated (Fig 3). However, a corresponding relationship was not observed between $t_{½}$ and $AUC_{0\text{-}12}$ of felodipine with grapefruit juice ($r = 0.02$, $P = .96$) or 2 mg ($r = 0.04$, $P = .91$), 6 mg ($r = 0.19$, $P = .57$), or 12 mg bergamottin ($r = 0.16$, $P = .64$).

Plasma bergamottin and 6′,7′-dihydroxybergamottin concentrations. Data from the 8 subjects who had measurable plasma bergamottin levels are shown in Table II. One volunteer (subject 7) had a

Table I. Pharmacokinetics of felodipine and its dehydrofelodipine metabolite after single dose administration of 5-mg extended-release tablet of felodipine with 250 mL water; 2 mg, 6 mg, or 12 mg bergamottin; or grapefruit juice (N = 11)

		Bergamottin			
	Water	*2 mg*	*6 mg*	*12 mg*	*Grapefruit juice*
Felodipine					
$AUC_{0\text{-}12}$ (nmol/L · h)	20.1 ± 4.4	22.8 ± 6.8	25.6 ± 7.3	26.7 ± 8.3*	29.8 ± 7.8**
$AUC_{0\text{-}24}$ (nmol/L · h)	34.1 ± 10.2	36.1 ± 10.5	39.0 ± 11.2	42.0 ± 12.9	46.0 ± 12.8**
C_{max} (nmol/L)	2.8 ± 0.5	3.6 ± 1.0*	3.7 ± 1.0**	3.9 ± 1.3*	5.2 ± 2.3**
t_{max} (h)	5.0 ± 3.9	5.1 ± 1.9	3.7 ± 1.2	4.5 ± 2.2	3.0 ± 1.4
$t_{½}$ (h)	22.9 ± 24.0	13.0 ± 9.9	12.9 ± 6.6	13.0 ± 6.2	12.8 ± 9.5
Dehydrofelodipine					
$AUC_{0\text{-}12}$ (nmol/L · h)	47.9 ± 11.8	53.3 ± 20.1	61.7 ± 23.4	63.1 ± 13.3**	60.8 ± 23.6**
$AUC_{0\text{-}24}$ (nmol/L · h)	68.7 ± 23.5	69.3 ± 23.0	86.3 ± 37.3	83.8 ± 15.5	77.2 ± 33.1
C_{max} (nmol/L)	9.3 ± 3.7	9.4 ± 3.2	10.6 ± 2.7	10.3 ± 2.6	12.4 ± 3.6*
t_{max} (h)	5.1 ± 4.2	4.5 ± 2.2	3.1 ± 1.3	3.2 ± 1.4	3.3 ± 1.8
$t_{½}$ (h)	7.5 ± 6.2	5.6 ± 1.5	6.3 ± 4.3	9.4 ± 10.9	4.8 ± 0.9
Dehydrofelodipine/felodipine					
$AUC_{0\text{-}12}$ (nmol/L · h)	2.5 ± 0.7	2.3 ± 0.6	2.4 ± 0.8	2.4 ± 0.4	2.1 ± 0.7

Data are presented as mean ± SD.
$AUC_{0\text{-}12}$, Area under plasma concentration–time curve from 0 to 12 hours; $AUC_{0\text{-}24}$, area under plasma concentration–time curve from 0 to 24 hours; C_{max}, peak concentration; t_{max}, time to reach peak concentration; $t_{½}$, terminal half-life.
*$P < .05$ for treatment comparisons with water as control.
**$P < .025$ for treatment comparisons with water as control.

single quantifiable bergamottin concentration at 0.5 hour after administration of grapefruit juice or 2 mg bergamottin. Six and eight volunteers had at least 1 quantifiable concentration and t_{max} of bergamottin ranging between 0.5 and 2.5 hours after ingestion of 6 mg and 12 mg bergamottin, respectively. The plasma levels of bergamottin after a 12-mg dose were variable and significantly higher in 1 subject. Mean C_{max} values of bergamottin among individuals after 6 mg bergamottin tended to be lower than those after 12 mg bergamottin. Two volunteers (subjects 6 and 7) had 4 detectable plasma concentrations of 6′,7′-dihydroxybergamottin that were below the limit of quantification (1 ng/mL). One volunteer (subject 8) had quantifiable plasma levels of 6′,7′-dihydroxybergamottin as shown in Table II.

Adverse events. All treatments were generally well tolerated. Adverse events were mild and resolved during the study day without medical intervention. They included headache, dizziness, fatigue, muscle cramps, and nausea. The number of events in each treatment group was as follows: control, 8; grapefruit juice, 19; 2 mg bergamottin, 12; 6 mg bergamottin, 8; and 12 mg bergamottin, 12.

DISCUSSION

To our knowledge, this is the first report to evaluate the effect of bergamottin as an active ingredient in grapefruit juice–drug interactions by administering it as a pure substance. Furanocoumarins have been known for some time to inhibit in vitro oxidative metabolism mediated by CYP3A4[22,25] and are considered to be the active ingredients responsible for the grapefruit juice effect.[15] However, previous clinical investigations assessing the role of furanocoumarins have administered impure fractions from grapefruit juice or tested the effect of the citrus juices, lime juice, or Seville (bitter) orange juice, which also contain certain furanocoumarins found in grapefruit juice.[15,29,30,33,38,39] An alternative, likely more desirable approach that was taken in this study was to test the clinical effect of a potentially important furanocoumarin by administering it as a pure substance to humans.

In this study a single oral dose of 2 mg bergamottin, approximating the amount in a normal-sized glass of grapefruit juice (1.7 mg), did augment the C_{max} of felodipine. Although the AUC of felodipine was not statistically increased, the high correlation between the change in C_{max} and AUC for felodipine among individuals indicated that the oral bioavailability of felodipine might be increased by 2 mg bergamottin. However, this was not apparent, most likely because the effect was markedly less than that observed with whole grapefruit juice. These results are in agreement with

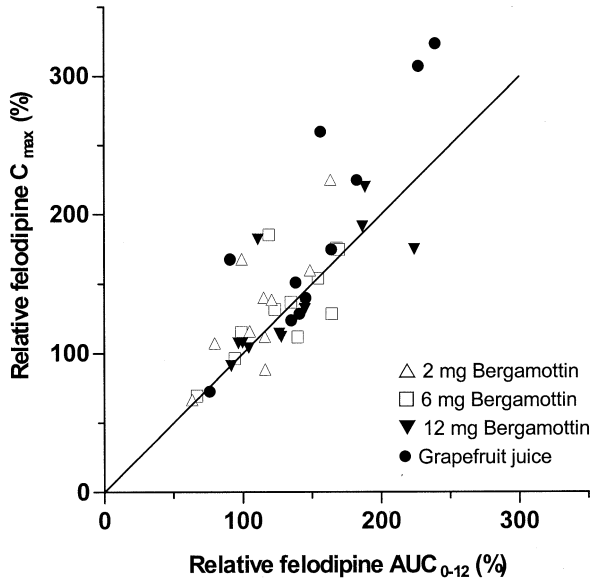


Fig 3. Relative felodipine maximal plasma drug concentration (C_{max}) (bergamottin or grapefruit juice relative to water expressed as percent) plotted against relative felodipine area under plasma concentration–time curve from 0 to 12 hours (AUC_{0-12}) for each individual with 250 mL grapefruit juice (*circles*) or 2 mg (*open triangles*), 6 mg (*squares*), or 12 mg bergamottin (*solid triangles*). The *diagonal line* represents the line of unity.

those obtained after administration of lime juice or grapefruit juice containing comparable amounts of bergamottin,[33] supporting a contribution of bergamottin to the grapefruit juice interaction with felodipine.

In this study 6 mg and 12 mg bergamottin had an effect that was not substantially greater than that of 2 mg bergamottin and less than that of grapefruit juice. With the assumption that bergamottin given as a pure substance has the same disposition as bergamottin in whole grapefruit juice, it would also appear that bergamottin is not the only active ingredient and that additional substances contribute to the magnitude of the interaction. Given that 2 mg bergamottin accounted for only a mean 30% of the grapefruit juice effect, these additional ingredients might be expected to contribute substantially to the interaction.

In a previous study grapefruit juice was separated by high-speed centrifugation and ultrafiltration into a particulate fraction, which contained all of the bergamottin, and a supernatant fraction, which contained most of the 6′,7′-dihydroxybergamottin.[29] Results with the particulate fraction indicated that bergamottin might be a major active constituent.[21,29] However, it should be noted the particulate material also contains trace amounts of at least 3 other furanocoumarins that are dimer derivatives of 6′,7′-dihydroxybergamottin and potent in vitro inhibitors of CYP3A4.[21,23,40] Thus these other furanocoumarins may contribute substantially to the clinical interaction between grapefruit juice and drugs.[21] Grapefruit juice coadministration with felodipine generally results in 1.5- to 3.5-fold increases in felodipine AUC.[3] In this study grapefruit juice increases in mean felodipine AUC (1.5-fold) and C_{max} (1.9-fold) were modest even though bergamottin concentrations in the juice were greater than average,[12,20,21] implicating a contribution of other active components and lower concentrations of active ingredients present in the brand of juice studied. Moreover, the in vitro observation that the inhibitory effect of furanocoumarins on the activity of CYP3A4 only approximates that of grapefruit juice when a combination of several furanocoumarins are simultaneously tested at highest concentrations suggested that the mechanism might involve an additive or synergistic interaction among active furanocoumarins.[12,21,23,40,41] Therefore it would be of interest to investigate the effect of a cocktail of furanocoumarins at concentrations similar to those present in grapefruit juice on in vivo drug disposition.

Although 2 mg bergamottin produced a modest mean increase in the oral bioavailability of felodipine, the magnitude of the interaction was highly variable among individuals. Certain individuals had more than a doubling in the plasma concentration of felodipine. Moreover, the magnitude of the effect was consistent within individuals with repeat administration of bergamottin. Thus the presence of bergamottin in fruit juices and possibly other food sources or herbal supplements might produce clinically significant drug interactions in certain susceptible individuals.

This variability of effect among individuals might be explained by variable bioavailability or altered disposition of bergamottin, possibly from low or inconsistent permeability into cells containing CYP3A4.[42] However, the measurement of bergamottin in plasma samples, particularly at the higher doses, indicates that this substance was likely accessible to intestinal and hepatic CYP3A4. Because felodipine is not a substrate of P-glycoprotein, it is unlikely that the previously reported in vitro inhibitory activity of bergamottin toward this efflux transporter could explain the inconsistent effect among subjects.[31,43] Previously, it was demonstrated that individuals with the highest baseline enterocyte content of CYP3A4 had the largest increase in felodipine bioavailability with grapefruit juice.[8] Thus

Table II. Individual pharmacokinetics (n = 8) of bergamottin and 6′,7′-dihydroxybergamottin after administration of grapefruit juice or 2 mg, 6 mg, or 12 mg bergamottin

	No. of samples containing BG or DHB†*				*C_{max} (ng/mL)*				*t_{max} (h)*			
		BG				*BG*				*BG*		
Subject No.	*GFJ*	*2 mg*	*6 mg*	*12 mg*	*GFJ*	*2 mg*	*6 mg*	*12 mg*	*GFJ*	*2 mg*	*6 mg*	*12 mg*
1	—	—	3	3	—	—	1.9	4.9	—	—	1.0	1.0
3	—	—	1	1	—	—	1.6	2.4	—	—	0.5	0.5
4	—	—	3	4	—	—	2.1	2.8	—	—	0.5	1.0
5	—	—	—	3	—	—	—	2.9	—	—	—	1.0
6	—	—	—	4	—	—	—	6.1	—	—	—	1.0
7	1	1	2	3	1.2	6.7	2.5	2.8	0.5	0.5	1.0	1.5
8	—	—	1	2	—	—	1.7	23	—	—	0.5	0.5
8†	—	—	—	—	3	—	—	—	5.9	—	—	0.5
9	—	—	2	4	—	—	2.7	2.4	—	—	1.0	2.5
Mean‡							2.1§	5.9‖			0.8§	1.1‖
SD							0.4	6.9			0.3	0.6

BG, Bergamottin; DHB, 6′,7′-dihydroxybergamottin; GFJ, grapefruit juice.
*Number of samples in which bergamottin was detected (ie, >1 ng/mL) (samples analyzed from 0 to 3 hours).
†Number of samples in which 6′,7′-dihydroxybergamottin was detected (ie, >1 ng/mL) (samples analyzed from 0 to 3 hours).
‡Mean and SD values are presented for bergamottin plasma levels.
§n = 6.
‖n = 8.

the most viable cause for the variability in the extent of the interaction with felodipine among individuals appears to be inherent variation in the enterocyte content of CYP3A4.

6′,7′-Dihydroxybergamottin was quantified in the plasma of 1 subject, and the peak concentration of this metabolite was rapid and occurred at the same time as that of the parent compound. Thus biotransformation of bergamottin to the dihydroxylated product appears to occur in vivo and to be rapid. Furanocoumarins are believed to act clinically by mechanism-based inactivation of CYP3A4. The plasma measurement of 6′,7′-dihydroxybergamottin suggested that the reactive substance might be a chemically unstable intermediate of these 2 substances, in addition to the proposed bioactivation of the furan ring.[44] Moreover, in vitro irreversible kinetic parameters for bergamottin indicate that an in vivo drug interaction with CYP3A4 substrates is either "likely" or "possible."[22,23,26,45] However, current predictive models for clinical drug interactions with irreversible enzyme inhibitors are still being developed and do not account for an interaction at the level of the enterocyte.[46]

Biotransformation of felodipine by CYP3A4 normally yields a single inactive metabolite, dehydrofelodipine. Previous studies show that grapefruit juice increased the AUC of felodipine and decreased the dehydrofelodipine/felodipine AUC ratio, suggesting inhibition of the primary metabolic pathway.[4] In this study neither grapefruit juice nor bergamottin decreased the dehydrofelodipine/felodipine AUC ratio to a statistically significant extent, possibly as a result of the insufficient magnitude of the increase in the AUC of felodipine as a result of the study population and the batch of grapefruit juice tested.

In summary, bergamottin administered as a pure substance enhanced the oral bioavailability of felodipine. However, the effect was substantially less than that produced by grapefruit juice even at markedly higher doses of bergamottin than normally present in the juice. Nevertheless, foods containing only bergamottin would likely cause a clinically relevant drug interaction in susceptible individuals. It appears probable that the interaction also involves other furanocoumarins present in whole grapefruit juice, possibly acting in combination by additive or synergistic mechanisms. Bergamottin has systemic availability and is metabolized to 6′,7′-dihydroxybergamottin in humans.

We acknowledge Dr Faans Steyn for statistical analysis of data.

Pfizer Inc has a patent (US 6,509,371) on compositions containing bergamottin for increasing the oral bioavailability of pharmaceutical agents, coinvented by Drs He and Hollenberg. Drs Goosen, Yu, Cohen, and Williams are employees of and have stock in Pfizer Inc. Dr He was an employee of Pfizer Inc and is currently employed by

Bristol-Myers Squibb. Dr Hollenberg has consulted for, received research funding from, and has stock in Pfizer Inc. Dr Woster is a coinventor of a patent on the synthesis and use of 6′,7′-dihydroxybergamottin (US 6,160,006). Ms Cillié and Drs Bailey, Rheeders, and Dijkstra have no conflict of interest.

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