

Grapefruit juice ingestion significantly reduces talinolol bioavailability

Objectives: Our objectives were to evaluate the effect of single and repeated grapefruit juice ingestion relative to water on the oral pharmacokinetics of the nonmetabolized and P-glycoprotein–transported drug talinolol in humans and to assess the potential impact of grapefruit juice ingestion on P-glycoprotein and intestinal uptake transporters.

Methods: The oral pharmacokinetics of 50 mg talinolol was determined with water, with 1 glass of grapefruit juice (300 mL), and after 6 days of repeated grapefruit juice ingestion (900 mL/d) in 24 healthy white volunteers. *MDR1* messenger ribonucleic acid and P-glycoprotein levels were measured in duodenal biopsy specimens obtained from 3 individuals before and after ingestion of grapefruit juice. Three commonly occurring polymorphisms in the *MDR1* gene were also assessed.

Results: A single glass of grapefruit juice decreased the talinolol area under the serum concentration–time curve (AUC), peak serum drug concentration (C_{max}), and urinary excretion values to 56% ($P < .001$), 57% ($P < .001$), and 56% ($P < .001$), respectively, of those with water. Repeated ingestion of grapefruit juice had a similar effect (44% to 65% reduction; $P < .01$). Single or repeated juice ingestion did not affect renal clearance, elimination half-life, or time to reach C_{max} (t_{max}). *MDR1* messenger ribonucleic acid and P-glycoprotein levels in duodenal biopsy specimens were not affected by grapefruit juice. *MDR1* genotypes (C1236T, G2677T/A, and C3435T) were not associated with altered talinolol pharmacokinetics.

Conclusion: Because both single and repeated ingestion of grapefruit juice lowered rather than increased talinolol AUC, our findings suggest that constituents in grapefruit juice preferentially inhibited an intestinal uptake process rather than P-glycoprotein. Moreover, grapefruit juice did not alter intestinal P-glycoprotein expression. (Clin Pharmacol Ther 2005;77:291-301.)

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Dietary constituents are increasingly recognized as important determinants of variable drug absorption

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from the gastrointestinal tract. Indeed, grapefruit juice is now widely acknowledged to be clinically important because its ingestion leads to inhibition of intestinal cytochrome P450 (CYP) 3A4 enzyme–mediated drug metabolism.¹⁻⁴ The action of grapefruit juice is rapid and significant, even after consumption of a single glass of juice. Moreover, grapefruit juice–mediated loss of enteric CYP3A is irreversible, and thus the duration of effect can exceed 24 hours.⁵⁻⁷ Grapefruit juice can cause enhanced drug absorption as a result of reduced first-pass intestinal metabolism of substrate drugs.

Recent studies indicate that juice–drug interactions are not limited to inhibition of drug-metabolizing enzymes; constituents in grapefruit juice may also affect drug absorption via modulation of transport processes.⁸ In the intestine 2 types of transport systems have been localized to the apical membrane of the enterocyte.^{9,10} Efflux transporters such as P-glycoprotein extrude

drugs from the enterocyte back into the intestinal lumen, thereby limiting drug absorption from the gastrointestinal tract.^{11,12} In addition, uptake transporters including members of the organic anion transporting polypeptide (OATP) family enable enterocyte drug influx, thereby enhancing oral drug bioavailability.¹³⁻¹⁵ Therefore the net absorption of a drug is likely dependent on the relative contribution of intestinal drug metabolism and both efflux and uptake drug transporters.

In a recent study we showed that grapefruit juice had a significant *in vitro* inhibitory effect on OATP-mediated drug uptake compared with P-glycoprotein-mediated efflux and produced a marked *in vivo* reduction in the oral bioavailability of the nonmetabolized and OATP- and P-glycoprotein-transported antihistamine fexofenadine.^{8,16} Currently, there are no clinical data regarding the effect on the nonmetabolized (<1%) β_1 -adrenergic receptor antagonist talinolol, which has been clearly determined to be a substrate for P-glycoprotein by use of polarized human intestinal cells and mice lacking *mdr1a/1b*-encoded P-glycoprotein.^{17,18} Talinolol is a long-acting, highly selective β_1 -antagonist without intrinsic sympathomimetic activity.¹⁹ The drug was introduced into clinical practice in 1975 and is frequently used in Germany and Eastern Europe to treat hypertension, coronary heart disease, and tachydysrhythmias at oral doses of 50 to 300 mg/d. With regard to β_1 selectivity, talinolol is comparable to metoprolol, and its pharmacokinetic characteristics are comparable to those of bisoprolol.¹⁹ Another randomized, double-blinded clinical study compared the effects of talinolol and atenolol on blood pressure in relation to lipid and glucose metabolic parameters. No difference was observed between the 2 drugs in terms of their antihypertensive effect in patients with mild to moderate hypertension.²⁰

Even though talinolol is a P-glycoprotein substrate and noted to have a pH-dependent intermediate lipid solubility and low water solubility,²¹ it is well absorbed, with an absolute oral bioavailability averaging $55\% \pm 15\%$, suggesting a role for active uptake transport.^{22,23} It is interesting that the oral coadministration of talinolol and verapamil resulted in reduced serum levels of talinolol, suggesting that verapamil affects an absorptive process because inhibition of P-glycoprotein would be predicted to increase the serum level of talinolol.^{18,24} Because we had previously determined that verapamil is also an inhibitor of OATP1A2 (previously called OATP-A), 1 or more OATP-type transporters are likely to be involved in the intestinal transport of talinolol.¹³

Accordingly, if grapefruit juice has a more potent inhibitory effect on intestinal uptake, relative to P-glycoprotein-mediated efflux, lower-than-predicted serum levels of the drug may be noted. Conversely, if grapefruit juice more specifically inhibits intestinal P-glycoprotein, a higher serum concentration of talinolol would result. Thus, to test such a hypothesis, we evaluated the effect of single and repeated grapefruit juice ingestion on talinolol oral pharmacokinetics in healthy volunteers in comparison with the effect of water. In addition, we measured intestinal *MDR1* messenger ribonucleic acid (mRNA) and P-glycoprotein levels in 3 individuals before and after ingestion of grapefruit juice. Furthermore, a potential role of polymorphisms in *MDR1* for talinolol disposition was also assessed.

METHODS

Study population and *MDR1* genotyping

Twenty-four healthy, nonsmoking men (age range, 19-32 years; weight range, 67-92 kg) were enrolled in the study. Each subject was noted to be in good health as assessed by a medical history, physical examination, routine laboratory testing (which included hematologic and serum chemical testing), and a standard 12-lead electrocardiogram (ECG). Only subjects with a normal PR interval, resting pulse rate greater than 45 beats/min, and systolic/diastolic blood pressure greater than 105/60 mm Hg were recruited. No subject had any significant illnesses within the preceding 16 days, was taking other medications, or had any history of cardiac, renal, hepatic, or gastrointestinal disease or of drug or alcohol abuse. All individuals provided written informed consent for the study, which had been approved by the Ethics Committee at the Medical Faculty Carl Gustav Carus, Technical University (Dresden, Germany), and by the German National Agency for Medicines (Bonn, Germany).

Genomic deoxyribonucleic acid (DNA) was prepared from venous blood samples by use of the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to the instructions of the manufacturer. Subjects were genotyped for the following 3 known *MDR1* single nucleotide polymorphisms that are most frequently observed in white subjects: exon 12, C1236T (Gly412Gly); exon 21, G2677T/A (Ala893Ser/Thr); and exon 26, C3435T (Ile1145Ile).²⁵ The genotype of *MDR1* was identified by single-strand conformational polymorphism analysis in exon 12 and by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis in exons 21 and 26 as previously described.^{26,27}

Experimental protocol

Grapefruit juice (Paradiso–Succo di pompelmo; Penny GmbH Deutschland, Cologne, Germany; 100% pure at a normal strength) was purchased from a Penny grocery market in Dresden, Germany. The same brand and lot number (7267045-K 7847) were used throughout the study. The amount of the grapefruit juice constituents naringin, dihydroxybergamottin, and bergamottin was measured by HPLC according to a previously published method,²⁸ with mean values of 712, 492, and 45 $\mu\text{mol/L}$, respectively. The juice constituents were assayed in triplicate, and the coefficients of variation of the standard curve were less than or equal to 5% at 85 $\mu\text{mol/L}$, 5.8% at 25 $\mu\text{mol/L}$, and 1.7% at 15 $\mu\text{g/mL}$ for naringin, dihydroxybergamottin, and bergamottin, respectively.²⁸ The pharmacokinetics of oral talinalol was determined after the administration of a 50-mg tablet of talinalol (Cordanum 50; AWD.pharma GmbH & Co KG, Dresden, Germany) at 24 hours on 3 different occasions during the study. The first tablet of talinalol was given on day 1 of the study with a 300-mL glass of water. The second tablet was taken on day 8 with the first 300-mL glass of regular-strength grapefruit juice. On days 9 through 14 of the study, 300 mL of grapefruit juice was ingested by each subject 3 times daily. The final tablet of talinalol was taken on day 15 with the 20th glass of grapefruit juice.

On days 1, 8, and 15, after a 10-hour overnight fast, peripheral venous blood (7.5 mL) was collected just before and at 20, 40, 60, 80, and 100 minutes and 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14, and 24 hours after talinalol administration. Urine was collected between 0 and 48 hours. For the first 3 hours after the drug was given, the subjects maintained a sitting position. They consumed a standardized meal 5 and 10 hours after drug administration. Consumption of beverages that contained caffeine or alcohol were not allowed for 2 days before and during the study period. Medications (including herbal and over-the-counter drugs) or grapefruit and other citrus or fruit juices were not permitted for 1 week before or during the study.

Proximal small-bowel mucosal biopsy specimens were obtained by upper intestinal endoscopy from 3 subjects who were randomly selected on study day 2 and after the last glass of grapefruit juice (day 16).

Safety monitoring. Adverse events were elicited from the volunteers by means of spontaneous reporting and specific questioning. Serial measurements of blood pressure (boso Oscillomat; BOSCH + SOHN GmbH, Jungingen, Germany) were taken and heart rate was recorded 3, 6, 12, and 24 hours after talinalol was

administered, and electrocardiographic tracings were recorded at 3, 10, and 24 hours after drug intake.

Electrocardiographic data. Twelve-lead ECGs were interpreted, and PR intervals were measured to record atrioventricular conduction delays. The ECG was recorded at a speed of 50 $\text{mm} \cdot \text{s}^{-1}$ (ECG AT-104 PC; Schiller AG, Baar, Switzerland).

Duodenal biopsy specimens

Endoscopy. Three subjects underwent upper intestinal endoscopy after fasting overnight. They were sedated with intravenous midazolam (Dormicum; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany), and a videoendoscope was used to obtain 6 mucosal biopsy specimens from the second portion of the duodenum. Four of the biopsy specimens were immediately snap-frozen in liquid nitrogen, and the other 2 were placed in RNA stabilization reagent (RNAlater; Qiagen, Hilden, Germany) and subsequently stored at -20°C .

P-glycoprotein Western blot analysis. Intestinal biopsy specimens were homogenized in homogenization buffer (100-mmol/L Tris base, 1-mmol/L ethylenediaminetetraacetic acid, 1-mmol/L 4-[2-aminoethyl]-benzenesulfonyl fluoride hydrochloride protease inhibitor [Pefa Bloc], 1 mg/L of leupeptin, and 1 mg/L of pepstatin) by use of a 2-mL conical Wheaton glass tube with a motor-driven Teflon pestle (1000 rpm for 1 minute). Pefa Bloc protease inhibitor was purchased from Roth (Karlsruhe, Germany), and leupeptin and pepstatin were purchased from Sigma (Deisenhofen, Germany). The total protein amount of the homogenates was determined according to the method of Smith et al.²⁹ Then 75 μg of the homogenates was prepared in 61.2-mmol/L Tris–hydrochloric acid (pH 6.8), 2% (wt/vol) sodium dodecyl sulfate, 5% (vol/vol) 2-mercaptoethanol, 5% (vol/vol) glycerol, and 0.02% (wt/vol) bromophenol blue and separated on 10% sodium dodecyl sulfate–polyacrylamide slab gels.³⁰ Transfer of proteins to nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) was carried out in a tank blotter semidry transfer cell (Trans-Blot SD Semi-Dry Transfer Cell; Bio-Rad Laboratories Inc, Hercules, Calif). The blot was then rinsed once with Tris-buffered saline solution (TBS) and incubated in blocking buffer (TBS with 5% nonfat dry milk and 0.1% polyoxyethylenesorbitan monolaurate [Tween 20; ICI America, Inc, Wilmington, Del]) for 1 hour. Thereafter the blot was incubated with a monoclonal anti-P-glycoprotein antibody, F4, in blocking buffer (Sigma) for 1 hour. The blot was washed 2 times with washing buffer (TBS with 0.1% Tween 20) and then incubated with peroxidase-labeled antimouse immunoglobulin G

in blocking buffer (Oncogene, Cambridge, Mass) for 30 minutes. The chemiluminescence signal (SuperSignal WestDura; Pierce, St Augustin, Germany) was measured with a CCD (charged coupled device) camera (Fuji LAS-1000; Raytest, Straubenhardt, Germany). L-MDR1 cell homogenates (5, 2.5, and 1 μg) were used as positive controls for P-glycoprotein (L-MDR1 cells were kindly provided by Dr Alfred Schinkel, The Netherlands Cancer Institute, Amsterdam, The Netherlands).

MDR1 and villin mRNA expression analysis. Intestinal biopsy specimens stored in RNAlater stabilization reagent were homogenized in RLT buffer (Qiagen) containing 1% 2-mercaptoethanol (Sigma) by sonication. Total ribonucleic acid from intestinal biopsy specimens was purified by use of a kit (RNeasy Mini Kit; Qiagen) according to the manufacturer's instructions. Complementary deoxyribonucleic acid (cDNA) synthesis of each sample was performed by use of a kit (*TaqMan* Reverse Transcription Kit; Perkin Elmer/Roche Molecular Systems, Inc, Branchburg, NJ). Reaction conditions were as follows: 10 minutes at room temperature, followed by 30 minutes at 48°C and another 5 minutes at 95°C. For the quantification of MDR1 mRNA, a detection system (ABI PRISM 7700 Sequence Detection System; Perkin Elmer Applied Biosystems, Foster City, Calif) was used as previously described.³¹ In brief, oligonucleotide primers and *TaqMan* probe were designed with Primer Express software (version 1.0) (Perkin Elmer Applied Biosystems) by use of the MDR1 sequence from the GenBank database (accession No. M14758). The forward primer sequence for *MDR1* was TAGAAGATCTGATGTC AAACATCACTAATAGA, and the reverse primer sequence for *MDR1* was GTGTATTTGCTCTC CAGCTGCCA. The probe sequence for *MDR1* labeled with the fluorophores 5'-carboxyfluorescein [FAM (5')] and 3'-carboxytetramethylrhodamine [TAMRA (3')] was AGGAAGACATGACCAGGTATGCCTAT TATTACAG. The *MDR1* mRNA expression was normalized to the mRNA expression of the enterocyte-specific expressed villin. The forward primer sequence for villin was CTGGCAACCTTAGGGACTGG, and the reverse primer sequence for villin was GTTAG CATTGAACACGTCCACTTT. The probe sequence for villin labeled with the fluorophores FAM (5') and TAMRA (3') was CCAGATCACTGCTGAGGTCA CAAGC. Reverse transcription-PCR were carried out in a 25- μL volume containing cDNA from 50 ng total ribonucleic acid, a PCR mix (*TaqMan* Universal PCR Master Mix; Perkin Elmer), and an MDR1-specific probe (5 $\mu\text{mol/L}$), as well as forward (5 $\mu\text{mol/L}$) and reverse (5 $\mu\text{mol/L}$) primers. The PCR conditions for

the PCR were 95°C denaturation for 15 seconds and 60°C primer extension for 40 cycles. The *MDR1* mRNA was calculated as a ratio of the amount of *MDR1* mRNA (nanograms)/villin mRNA (nanograms).

Talinolol assay

Blood samples were collected in plastic tubes (S-Monovetten; Sarstedt AG & Co, Nümbrecht, Germany) and immediately centrifuged. Urine volumes were measured. Serum and urine samples were immediately frozen at -20°C until assays were obtained. Serum and urine concentrations were analyzed for unchanged talinolol by a specific and sensitive HPLC method as previously published.²² In the current study the lower limit of quantitation of the talinolol assays was 7 ng \cdot mL⁻¹ in serum and 200 ng \cdot mL⁻¹ for the urine samples, with a coefficient of variation lower than 10% for 6 repeated measurements, respectively. The within-day and between-day precisions were greater than 7.5% and greater than 9.6%, respectively. The accuracy ranged from 98.3% to 104% for the mean of 6 repeated measurements and from 89.3% to 110.8% for single values.

Pharmacokinetic and statistical evaluation

Serum talinolol concentrations were analyzed by use of a noncompartmental model. The terminal log-linear phase of the serum talinolol concentration-time profile was identified visually for each subject. 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 of the log-linear phase ($t_{1/2}$) was calculated as 0.693/ k_e . The area under the serum drug concentration-time profile (AUC) from 0 to 24 hours [AUC(0-24)] was calculated by use of the linear trapezoidal method. The AUC from 24 hours to infinity [AUC(24- ∞)] was determined by dividing the final talinolol serum concentration by k_e , and the AUC from 0 hours to infinity [AUC(0- ∞)] was the sum of AUC(0-24) and AUC(24- ∞). The peak concentration in serum (C_{max}) and the time to reach C_{max} (t_{max}) were obtained directly from the experimental data. The oral clearance (CL_{oral}) was determined by as follows: $\text{Dose}_{\text{oral}}/\text{AUC}(0-24)_{\text{oral}}$. Urinary excretion of unchanged talinolol from 0 to 48 hours (A_e) was calculated as urinary concentration multiplied by volume. The renal clearance of talinolol (CL_R) was calculated from A_e for 0 to 24 hours divided by AUC(0-24).

Descriptive and comparative statistics were calculated by use of SPSS for Windows, version 11.5 (SPSS Inc, Chicago, Ill). Comparison of mean talinolol pharmacokinetic indices after water and single or recurrent

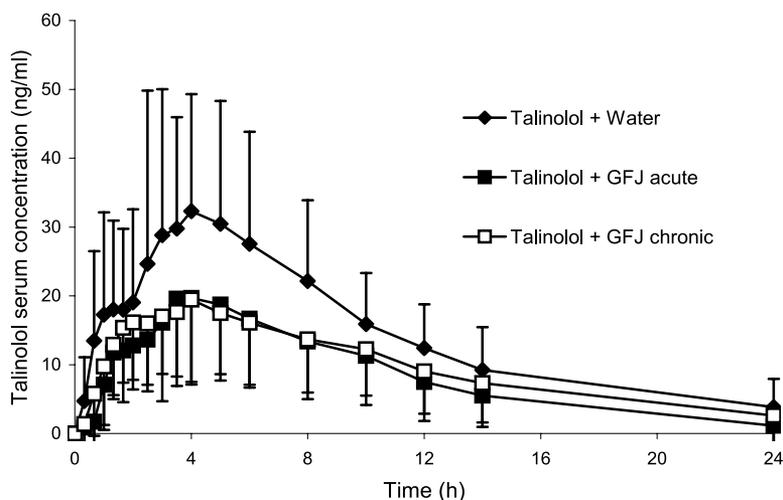


Fig 1. Mean (\pm SD) serum talinalol concentration–time profiles for subjects ($N = 24$) after orally administered talinalol (50 mg) with 300 mL of water, with 300 mL of grapefruit juice (GFJ acute), or after ingestion of 300 mL of grapefruit juice 3 times daily for 6 days (GFJ chronic).

ingestion of grapefruit juice was done by use of a paired 2-tailed *t* test, which assumes normally distributed data, or the nonparametric Wilcoxon test, as appropriate. Linear regression was used to examine possible interrelationships between parameter values. The F test (ANOVA) was used to analyze intersubject and intra-subject effects among the different *MDR1* genotypes. In addition, to compare the pharmacokinetic results between the genotypes on the 3 study days (water and single and recurrent grapefruit juice intake), the Kruskal-Wallis test, Jonckheere-Terpstra test, and median test were used, as appropriate. A value of $P < .05$ was considered statistically significant. The data are expressed as mean value \pm SD.

RESULTS

Grapefruit juice effect on talinalol pharmacokinetics

Grapefruit juice markedly lowered serum talinalol concentration compared with water (Fig 1). The pharmacokinetic values for talinalol disposition calculated on day 1 (1 glass of water), day 8 (single glass of grapefruit juice), and day 15 (20th glass of grapefruit juice) are shown in Table I. The first glass of grapefruit juice (short-term effect) decreased the mean talinalol AUC over a 24-hour period and C_{max} values to 56% ($P < .001$) and 57% ($P < .001$), respectively, and increased CL_{oral} to 162% ($P < .001$) of those for water. Talinalol t_{max} and $t_{1/2}$ were not affected. The 48-hour cumulative excretion of talinalol into urine was reduced to 56% of that observed with water ($P < .001$) without alteration in renal clearance. There was a significant

correlation between the baseline AUC and the decrease in AUC of talinalol by grapefruit juice (Fig 2); individuals with the highest talinalol AUC with water had the greatest decrease after grapefruit juice ingestion.

The 20th glass of grapefruit juice (long-term effect) reduced talinalol AUC and C_{max} to 64% ($P < .01$) and 57% ($P < .01$), respectively, and increased CL_{oral} to 223% ($P < .05$) of those for water. Recurrent grapefruit juice ingestion did not alter t_{max} and $t_{1/2}$. The cumulative excretion of talinalol into urine was reduced to 44% of that observed with water ($P < .001$); renal clearance was not affected.

There was no significant difference in talinalol pharmacokinetics after the first glass compared with continued ingestion of grapefruit juice (20th glass).

MDR1 mRNA and P-glycoprotein expression

Grapefruit juice did not significantly affect the amount of *MDR1* mRNA in the duodenal biopsy samples (normalized to villin) of the 3 investigated volunteers, as follows: $2.73 \cdot 10^{-5} \pm 2.7 \cdot 10^{-6}$ ng *MDR1*/ng villin mRNA before juice intake versus $2.89 \cdot 10^{-5} \pm 4.3 \cdot 10^{-6}$ ng *MDR1*/ng villin mRNA after repeated juice ingestion. Furthermore, 6 days of normal-strength grapefruit juice intake (900 mL/d) did not consistently alter mean P-glycoprotein level in the gastrointestinal biopsies specimens (Fig 3).

MDR1 genotype

The allele and genotype frequency distribution of the 3 polymorphisms of the *MDR1* gene (Table II) of the

Table I. Serum and urinary pharmacokinetics of 50 mg talinolol in 24 male subjects with 300 mL of water (day 1), after the first 300-mL glass of grapefruit juice (day 8), or after 6 days of thrice-daily ingestion of 300 mL of grapefruit juice (day 15; 20th glass of grapefruit juice)

	Water	Grapefruit juice	
		Single ingestion	Recurrent ingestion
Serum talinolol			
AUC(0-24) (ng · h/mL)	329 ± 175	183 ± 95***	209 ± 133**
AUC(0-∞) (ng · h/mL)	427 ± 192	301 ± 149*	310 ± 185*
C _{max} (ng/mL)	45 ± 24	26 ± 12***	26 ± 13**
t _{max} (h)	3.3 ± 1.5	3.9 ± 1.1	4.1 ± 2.2
t _{1/2} (h)	9.1 ± 4.1	11.0 ± 10.4	9.0 ± 4.4
CL _{oral} (0-24) (mL/min)	3501 ± 2518	5687 ± 2751**	7823 ± 11,230*
Urinary talinolol			
A _e (0-48) (mg)	4.5 ± 1.9	2.5 ± 2.0***	2.0 ± 0.9***
CL _R (0-24) (mL/min)	220 ± 102	190 ± 104	252 ± 374

Data are expressed as mean ± SD. There was no statistical difference between single and recurrent juice ingestion.

AUC(0-24), Area under serum concentration–time curve from 0 to 24 hours; AUC(0-∞), serum area under concentration–time curve from 0 hours extrapolated to infinity; C_{max}, peak serum drug concentration; t_{max}, time to reach peak serum drug concentration; t_{1/2}, elimination half-life; CL_{oral}, oral clearance from 0 to 24 hours; A_e, urinary excretion of unchanged talinolol from 0 to 48 hours; CL_R, renal clearance from 0 to 24 hours.

**P* < .05 for comparison of difference between juice and water ingestion.

***P* < .01 for comparison of difference between juice and water ingestion.

****P* < .001 for comparison of difference between juice and water ingestion.

study subjects (N = 24) was comparable to that seen in 686 unrelated white subjects.^{25-27,32} The 3 *MDR1* genotypes in exon 12 (C1236T, Gly412Gly), exon 21 (G2677T/A, Ala893Ser/Thr), and exon 26 (C3435T, Ile1145Ile), as well as the combination of 1 or more variant *MDR1* alleles in exons 12, 21, and 26, did not appear to be associated with altered pharmacokinetic characteristics of talinolol; the appropriate AUC(0-24) values are shown in Table II.

Hemodynamics

There were no clinically significant differences in PR intervals, heart rate, and blood pressure after oral administration of 50 mg talinolol given with water or grapefruit juice (data not shown).

DISCUSSION

In this study a single glass of normal-strength grapefruit juice markedly lowered serum talinolol concentrations compared with water whereas recurrent juice ingestion did not further enhance this effect. Furthermore, the *MDR1* mRNA and P-glycoprotein levels in the duodenal mucosa of 3 volunteers were not significantly altered by grapefruit juice.

The β-adrenergic receptor antagonist talinolol has been shown to be a P-glycoprotein substrate by use of polarized human intestinal cells and mice lacking *mdr1a/1b*-encoded P-glycoprotein.^{17,18} In humans a steady-state intestinal perfusion method has revealed

secretion of talinolol after an orally or intravenously administered dose of the drug against a concentration gradient into the gut lumen,²¹ and impairment of talinolol secretion was observed after coadministration with the P-glycoprotein inhibitor *R*-verapamil; both of these findings support that talinolol is intestinally transported by P-glycoprotein.^{33,34} Moreover, we observed that coadministration of verapamil reduced the oral talinolol serum levels in P-glycoprotein-null mice compared with wild-type mice,¹⁸ and a comparable finding of a reduction in talinolol AUC after oral verapamil administration was also observed in humans; both of these findings suggest inhibition of an intestinal uptake transport system.²⁴ When the normally negligible metabolism of talinolol is considered, these findings indicate that both intestinal P-glycoprotein efflux and intestinal uptake transporters such as members of the OATP transporter family are key determinants of talinolol disposition.²²

Grapefruit juice significantly decreased C_{max}, AUC, and cumulative urinary excretion of talinolol without prolongation of t_{1/2} or alteration in CL_R. These data suggest that the observed increase in oral clearance is likely a result of a decrease in oral bioavailability. This is in contrast to the majority of reported grapefruit juice–drug interactions, which result in elevated drug bioavailability related to mechanism-based inhibition of intestinal CYP3A4.^{2,8} However, recent studies have reported decreased oral bioavailability of both the an-

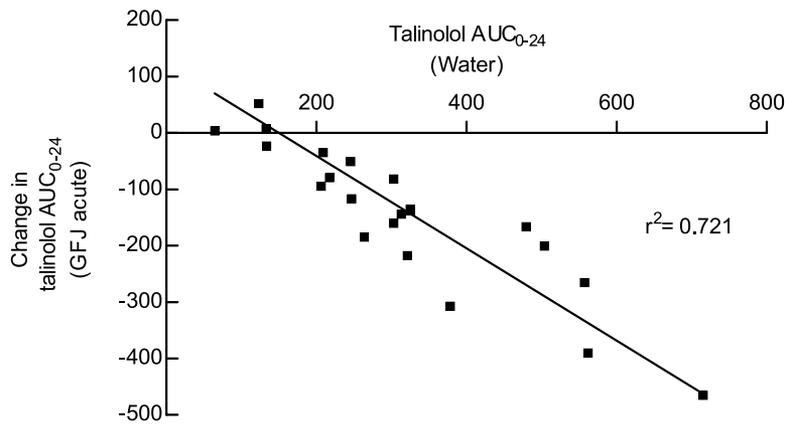


Fig 2. Change in area under serum talinolol concentration–time curve from 0 to 24 hours (AUC_{0-24}) with 300 mL of grapefruit juice (GFJ acute) plotted against serum talinolol AUC_{0-24} with water for each individual.

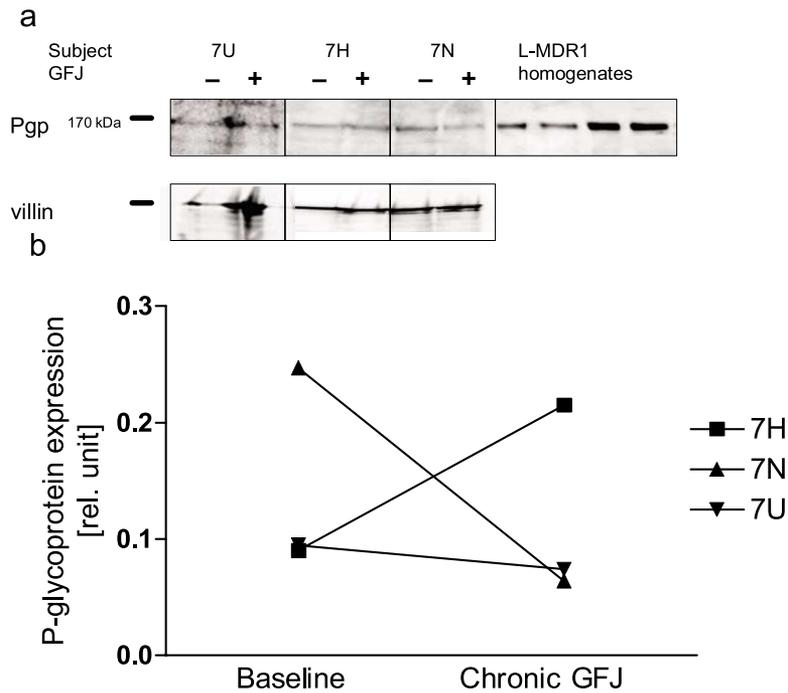


Fig 3. a, Western blot of P-glycoprotein (Pgp) expression normalized by villin in gastrointestinal biopsy specimens from 3 healthy volunteers before (–) and after (+) 6 days of recurrent grapefruit juice ingestion (300 mL of normal-strength juice 3 times daily). Intestinal biopsy homogenates (75 μ g total protein) were loaded on each lane; 0.5, 1.0, 2.5, and 5.0 μ g of L-MDR1 cell homogenates (kindly provided by Dr Alfred Schinkel) were used as calibration standards. The calibration curve was calculated by use of the 170-kd bands. **b,** Densitometric calculation values of the individual P-glycoprotein expression in 3 volunteers (7H, 7N, and 7U) before (baseline) and after (chronic GFJ) 6 days of recurrent grapefruit juice ingestion; the P-glycoprotein content was calculated from the intensities of the 170-kd band.

Table II. Results of *MDR1* genotyping and mean talinolol AUC(0-24) (\pm SD) after water (baseline), ingestion of 1 glass of grapefruit juice, or repeated grapefruit juice intake in current study (N = 24) in comparison with review frequency data (reference 25)

Location	cDNA position	Nucleotide change-allele	Effect	Allele frequency		Genotype
				Current study	Reference 25	
Exon 12	1236	C	412Gly	58.3	59.0	C/C
		T	412Gly	41.7	41.0	C/T
Exon 21	2677	G	893Ala	60.4	56.5	G/G
		T	893Thr	39.6	41.6	G/T
						T/T
		A	893Ser	0.0	1.9	G/A
Exon 26	3435	C	1145Ile	47.9	46.1	C/C
		T	1145Ile	52.1	53.9	C/T
						T/T
						A/A

cDNA, Complementary deoxyribonucleic acid.

tihistamine fexofenadine (an OATP and P-glycoprotein substrate) and the β -adrenergic receptor antagonist celiprolol (a P-glycoprotein substrate) when taken with grapefruit juice.^{13,16,35,36} Because biotransformation of these drugs is minimal, the primary effect by grapefruit juice appeared to involve inhibition of intestinal drug uptake by OATP transporters rather than efflux transport by P-glycoprotein as a likely mechanism for this interaction. Similarly, preferential impairment of intestinal OATP transport by grapefruit juice may be the explanation for a reduction in the oral bioavailability of talinolol. Constituents such as the furanocoumarins 6',7'-dihydroxybergamottin and bergamottin and the bioflavonoid naringin have been shown to produce potent in vitro inhibition of OATP uptake transporters versus P-glycoprotein at concentrations many-fold less than normally present in grapefruit juice. Thus these substances may directly inhibit intestinal OATPs, reduce the amount of talinolol taken up into the enterocyte, and effectively limit the amount of drug available for P-glycoprotein-mediated efflux back into the intestinal lumen.⁸ Moreover, grapefruit juice ingestion did not change *MDR1* mRNA or P-glycoprotein levels, confirming findings by Lown et al¹ and suggesting a lack of clinical effect on this transporter. Nevertheless, given that *MDR1* mRNA and P-glycoprotein levels were measured in only 3 volunteers in whom high interindividual variability was observed, additional clinical studies are needed to confirm the absence of grapefruit effect on intestinal P-glycoprotein expres-

sion. Currently, the effect of grapefruit juice on the expression of OATP transporters is unknown.

The weak base talinolol (pK_a [negative logarithm of the acid ionization constant] 9.4) might also be a potential substrate of polyspecific organic cation transporters (OCTs), because there is in vitro evidence that β -adrenergic receptor antagonists such as metoprolol are substrates of OCT2.³⁷ OCT1 and OCT2 are expressed in the basolateral membrane of epithelial cells of major excretory organs and transport a broad range of substrates, including drugs, toxins, and endogenous compounds.³⁸⁻⁴⁰ However, little is known regarding OCT expression in humans, in particular in the human intestine, and to our knowledge, there are no data about the potential effect of grapefruit juice on OCT. Given that grapefruit juice lowers the intestinal pH, the cationic form of talinolol might increase; this effect may be related to the observed findings in this study.

Recent in vitro data have shown that grapefruit juice and its components also interact with the efflux transporter multidrug resistance-associated protein (MRP) 2,⁴¹ which has been reported to be expressed in the human small intestine.⁴² Like P-glycoprotein, MRP2 is thought to facilitate the efflux of substrate drugs from organs such as the liver, intestine, and kidney.⁴³ There is evidence that talinolol is also a substrate of MRP2⁴⁴; therefore grapefruit juice-mediated inhibition of MRP2 cannot be excluded. However, inhibition of intestinal MRP2 or P-glycoprotein by grapefruit juice would be predicted to increase talinolol bioavailability, opposite

Genotype frequency		AUC(0-24) (ng · h/mL)		
Current study (N)	Reference 25	Baseline	Single ingestion of grapefruit juice (current study)	Recurrent ingestion of grapefruit juice (current study)
37.5 (9)	34.4	390.6 ± 161	224.9 ± 116	245.9 ± 160
41.7 (10)	49.2	322.4 ± 210	170.2 ± 82	206.0 ± 135
20.8 (5)	16.4	229.2 ± 71	131.9 ± 41	148.7 ± 35
41.7 (10)	30.9	358.0 ± 184	209.2 ± 121	222.9 ± 168
37.5 (9)	49.2	351.0 ± 201	181.5 ± 79	227.2 ± 124
20.8 (5)	16.1	229.2 ± 71	131.9 ± 41	148.7 ± 35
0.0	2.0	—	—	—
0.0	1.8	—	—	—
0.0	0.0	—	—	—
20.8 (5)	20.8	364.9 ± 111	154.9 ± 32	205.3 ± 186
54.2 (13)	50.5	368.4 ± 209	215.8 ± 116	238.9 ± 137
25.0 (6)	28.6	211.9 ± 75	134.3 ± 42	147.5 ± 35

to the finding observed in our study. Because talinalol absorption occurs in the proximal portion of the small intestine,²¹ an increase in gastrointestinal motility is another consideration that could be associated with lower talinalol absorption. It should be noted, however, that in healthy volunteers oral coadministration of 50 mg talinalol with the antibiotic erythromycin (2 g), a known P-glycoprotein inhibitor and a known enhancer of gut motility, significantly increased the talinalol serum AUC compared with the AUC of talinalol plus placebo.^{44a} Therefore it seems less likely that an increase in gastrointestinal motility is a major cause for the observed decrease in talinalol absorption in our study. Moreover, to our knowledge, there are no data regarding the effect of grapefruit juice on gastrointestinal motility.

In contrast, concomitant grapefruit juice enhanced oral talinalol bioavailability in rats.⁴⁵ The most prominent differences between the control and grapefruit juice–treated rats were a nearly 2-fold increase in C_{max} values, increases of 53% and 34% in AUC, and decreases of 53% and 21% in oral clearance for *S*- and *R*-talinalol, respectively, and no effect on $t_{1/2}$. Inhibition of P-glycoprotein–mediated intestinal efflux was thought to contribute to the increase in the bioavailability of the drug after grapefruit juice intake. One possible explanation for this opposite finding would be pH dependence of the juice-transporter interaction. In the rat study, grapefruit juice had been adjusted to pH 7, whereas in our investigation in humans no modification was made to the pH of the normal-strength juice. It is also possible that human OATPs or other uptake transporters responsible for talinalol uptake may be more

susceptible to the inhibitory effects of grapefruit juice. It is well known that there are marked species-related differences in the cDNA homology and substrate specificity between rat and human OATPs.¹⁴

We did not observe any association between the 3 *MDR1* genotypes and talinalol pharmacokinetic profiles. Similar findings for talinalol were made in another study that investigated the *MDR1* variants in exons 21 (G2677T/A) and 26 (C3435T), as well as 7 additional polymorphisms, in healthy white subjects; no influence of *MDR1* genotypes on duodenal expression of P-glycoprotein and disposition of oral and intravenous talinalol was observed.⁴⁶ Controversial results have been reported for a potential impact of *MDR1* genotypes on the disposition of the P-glycoprotein and OATP substrates digoxin and fexofenadine.^{26,32,47-50} Excellent reviews about the functional impact of *MDR1* polymorphisms have been published.^{25,51}

In summary, single or repeated ingestion of grapefruit juice was found to markedly reduce the oral bioavailability of the β_1 -adrenergic receptor antagonist talinalol, whereas the short-term and long-term grapefruit juice effect was similar. The results of this study provide further support that talinalol, a well-defined P-glycoprotein substrate that does not undergo significant biotransformation, is likely to depend on intestinal drug uptake processes. Predominant inhibition of an intestinal OATP family member by grapefruit juice rather than inhibition of intestinal P-glycoprotein would be associated with decreased talinalol serum levels. Additional studies relating to the characterization of intestinal uptake transporter for talinalol are

needed to more fully delineate the molecular basis of grapefruit juice effect.

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