

Pomegranate Juice Does Not Impair Clearance of Oral or Intravenous Midazolam, a Probe for Cytochrome P450-3A Activity: Comparison With Grapefruit Juice

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The effect of pomegranate juice (PJ) or grapefruit juice (GFJ) on CYP3A activity was studied *in vitro* and in healthy human volunteers. In human liver microsomes, the mean 50% inhibitory concentrations (IC_{50}) for PJ and GFJ versus CYP3A (triazolam α -hydroxylation) were 0.61% and 0.55%, (v/v) respectively, without preincubation of inhibitor with microsomes. After preincubation, the IC_{50} for PJ increased to 0.97% ($P < .05$), whereas the IC_{50} for GFJ decreased to 0.41% ($P < .05$), suggesting mechanism-based inhibition by GFJ but not PJ. Pretreatment of volunteer subjects ($n = 13$) with PJ (8 oz) did not alter the elimination half-life, volume of distribution, or clearance of intravenous midazolam (2 mg). Administration of PJ

also did not affect C_{max} , total area under the curve (AUC), or clearance of oral midazolam (6 mg). However, GFJ (8 oz) increased midazolam C_{max} and AUC by a factor of 1.3 and 1.5, respectively, and reduced oral clearance to 72% of control values. Thus, PJ does not alter clearance of intravenous or oral midazolam, whereas GFJ impairs clearance and elevates plasma levels of oral midazolam.

Keywords: cytochrome P450-3A; midazolam; grapefruit juice; pomegranate juice; *in vitro* metabolism; drug interactions

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Pomegranates (*Punica granatum*) have been used for centuries in traditional Chinese medicine as antimicrobial and anti-inflammatory agents.¹ The health benefits of this fruit are believed to be due to its high polyphenol content, since it is rich in antioxidants such as ellagic acid, punicalagin, and gallic acid.¹⁻⁴ The popularity of pomegranate juice increased significantly in the United States after laboratory and clinical studies suggested that its consumption could decrease the risk of cardiovascular

disease,⁵ suppresses prostate⁶ and breast cancers,⁷ and it might even augment the effect of HIV treatment regimens.⁸

Pharmacokinetic drug interactions involving citrus products have received considerable attention in the last 15 years. In early studies as far back as 1989, it was demonstrated that grapefruit juice increases the bioavailability of the calcium channel antagonists felodipine and nifedipine in humans.^{9,10} Many subsequent studies have indicated that the mechanism of the interaction mainly involves inhibition of enteric cytochrome P450-3A (CYP3A) isoforms by natural substances contained in grapefruit juice.¹¹⁻¹⁵ These compounds are largely mechanism-based inhibitors of CYP3A, in that they irreversibly deactivate the enzyme. Recovery of CYP3A function through enzyme regeneration requires approximately 3 days after consumption of grapefruit juice.¹⁶⁻¹⁹ One compound that appears to contribute to the observed inhibition is 6,7'-dihydroxybergamottin (DHB),^{16,20-30} a naturally

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occurring furanocoumarin found in grapefruit juice. Other furanocoumarin derivatives, including bergamottin itself and compounds formed by dimerization of DHB and/or bergamottin, might also contribute to the inhibitory effect of grapefruit juice.^{16,20-30} Clinical studies also demonstrate that grapefruit juice inhibits enteric CYP3A more than hepatic CYP3A.³¹⁻³⁴ Therefore, the consumption of grapefruit juice primarily affects the clearance of orally administered drugs which normally undergo significant metabolism by enteric CYP3A.¹¹ Thus, only a limited subset of compounds is subject to clinical interaction with grapefruit juice. Furthermore, drugs given by any route other than oral are unlikely to be affected.³¹⁻³⁴

Recent reports suggest that other fruit juices can also inhibit CYP 3A activity. Hidaka et al³⁵ reported that among tropical fruit juices, the most significant *in vitro* inhibitors of CYP3A (at concentrations of 5% in the incubation mixture) were grapefruit juice (85% inhibition), papaw juice (88% inhibition), pomegranate juice (97% inhibition), and star fruit juice, which almost completely inhibited the enzyme. Pomegranate juice also impaired CYP3A activity *in vivo* in Wistar rats. However, only enteric CYP3A was affected, and the extent of inhibition was comparable to that of grapefruit juice.³⁶ Currently, there are few studies investigating whether the interaction between pomegranate juice and CYP3A substrate drugs could be clinically significant in humans. Anecdotal case reports in the literature suggest the possibility of an interaction,³⁷ but the number of adequately controlled pharmacokinetic studies is small.³⁸

The first objective of this study was to examine inhibition of CYP3A by pomegranate juice *in vitro* in human microsomes using triazolam as an index substrate. The second objective was to examine the interaction of pomegranate juice with midazolam, an established probe compound used to profile CYP3A activity in healthy human volunteers. In the clinical study, inhibition was examined using both oral and intravenous midazolam in order to compare the effects on hepatic and enteric CYP3A. Grapefruit juice was used as a positive control for the inhibition in both the *in vitro* and clinical studies.

METHODS

In Vitro Studies

Liver samples from 4 individual human donors with no known liver disease were provided by the International Institute for the Advancement of Medicine (Exton, Pa); the Liver Tissue Procurement

and Distribution System, University of Minnesota (Minneapolis, Minn); or the National Disease Research Interchange (Philadelphia, Pa). All samples were of the CYP2D6 and CYP2C19 normal-metabolizer phenotype, based on prior *in vitro* phenotyping studies.

Microsomes were prepared by ultracentrifugation; microsomal pellets were suspended in 0.1 mol/L potassium phosphate buffer containing 20% glycerol and were stored at -80°C until use.³⁹ Pomegranate juice was obtained from PomWonderful (Los Angeles, Calif), and grapefruit juice manufactured by Ocean Spray (Lakeville-Middleboro, Mass) was purchased from a retail source.

Incubation mixtures (250 μL) contained 50 mmol/L phosphate buffer, 5 mmol/L magnesium, 0.5 mmol/L nicotinamide adenine dinucleotide phosphate, and an isocitrate/isocitric dehydrogenase regenerating system. The CYP3A index substrate, triazolam (250 $\mu\text{mol/L}$), was added to a series of incubation tubes along with 0, 0.05, 0.1, 0.25, 0.5, 1, and 2.5% (volume/volume) juice. Ketoconazole (0.1 μM) was also studied as a positive control.

Reactions were initiated by the addition of microsomal protein (0.1-0.25 mg/mL). After a 20-minute incubation duration at 37°C , reactions were stopped by cooling on ice and the addition of 100 μL of acetonitrile. Internal standard (phenacetin) was added, the incubation mixture was centrifuged, and the supernatant was transferred to an autosampling vial for high-performance liquid chromatography (HPLC) analysis. The mobile phase consisted of a combination of acetonitrile (20%), methanol (10%), and 10 mmol/L phosphate buffer (70%). The analytic column was stainless steel, 15 cm \times 3.9 mm, containing reverse-phase C18 Novapak (Waters Associates, Milford, Mass). Column effluent was monitored by ultraviolet absorbance at 220 nanometers.^{39,40} Studies were also repeated with a 20-minute period of preincubation of inhibitor with microsomes prior to the addition of substrate.

Reaction velocities with coaddition of inhibitor were expressed as a percentage ratio (R_v) of the control velocity with no inhibitor present. The relationship of R_v to inhibitor concentration was analyzed by nonlinear regression to determine the concentration producing a 50% decrement in reaction velocity (IC_{50}).⁴⁰

Clinical Study

Subjects

The study protocol and consent form were reviewed and approved by the institutional review board serving ProMedica Clinical Research Center, the site at

which the pharmacokinetic study was performed. All subjects provided written informed consent prior to participation. Fifteen healthy male volunteers were enrolled in the study. They were nonsmokers, free of medical disease, and taking no medications. Screening procedures included medical history, physical examination, hematologic profile, blood chemistries, urinalysis, and HIV and hepatitis positivity.

Study Design and Procedures

Subjects participated in a randomized, single-dose, 5-way crossover study, with 1 week elapsing between trials. The 5 treatment groups were:

1. Intravenous (IV) midazolam (2 mg) preceded by water
2. Oral midazolam (6 mg) preceded by water
3. IV midazolam (2 mg) preceded by pomegranate juice
4. Oral midazolam (6 mg) preceded by pomegranate juice
5. Oral midazolam (6 mg) preceded by grapefruit juice

Subjects were admitted to the study unit on the evening before each trial and received 8 oz of the fruit beverage or water, as appropriate. On the morning of the trial (7 AM), they received a standardized light breakfast and a second 8 oz of beverage or water. Oral or intravenous midazolam was administered at 8 AM. Blood samples were drawn prior to dosage and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hours after midazolam dosage. For the intravenous midazolam trials, an additional sample was drawn at 15 minutes. Blood samples were centrifuged, and the plasma separated and frozen until assay.

Analysis of Plasma Samples

Plasma concentrations of midazolam and its principal metabolite, 1-hydroxy-midazolam, were determined by liquid chromatography/mass spectrometry (LC/MS). After the addition of 200 ng of desmethyldiazepam as the internal standard, plasma samples (0.5 mL) from study subjects, along with calibration standards containing drug-free control plasma and various known concentrations of midazolam and 1-hydroxy-midazolam (0-100 ng/mL), were extracted with hexane/dichloromethane (70:30) using a vortex mixer. The organic extracts were separated, evaporated to dryness, reconstituted in 0.2 mL of mobile phase, and transferred to LC/MS autosampler vials; 25 μ L was injected onto the LC/MS.

Midazolam, 1-hydroxy-midazolam, and the internal standard (desmethyldiazepam) were separated and quantified using a Thermo Finnigan LCQ Deca XP Max HPLC/Mass Spectrometry system (version 1.3

SR1 SP2; Thermo Electron, Somerset, NJ), including an atmospheric pressure chemical ionization source operated in the positive-ion mode.⁴¹ The mobile phase consisted of 40% ammonium acetate (10 mmol/L, pH 4.3), 40% acetonitrile, and 20% methanol, at a flow rate of 0.35 mL/min, through a 150 \times 3-mm Synergi Fusion RP C18 column (Phenomenex, Torrance, Calif). For mass spectrometric analysis, the following atmospheric pressure chemical ionization inlet conditions were selected: source gas, nitrogen; vaporizer temperature, 400°C; discharge current, 3 μ A; capillary voltage, -13 V; and capillary temperature, 175°C.

Analytes were quantified via selective positive-ion monitoring of mass-to-charge ratios of 270.6 to 271.6 for desmethyldiazepam, 325.6 to 326.6 for midazolam, and 341.6 to 342.6 for 1-hydroxy-midazolam, with an expected peak retention time of 3.3 minutes for desmethyldiazepam, 3.8 minutes for midazolam, and 2.7 minutes for 1-hydroxy-midazolam.

Calibration curves (area ratio versus midazolam or 1-hydroxy-midazolam concentrations) were linear, and intercepts did not differ from zero. The sensitivity limit of the assay was 1 ng/mL, which was the lowest point on the calibration curve. At 1 ng/mL, the within-day coefficients of variation (CV) were 11.9% for midazolam and 15.6% for 1-hydroxy-midazolam. At 10 ng/mL, values were 15.5% for midazolam and 7.2% for 1-hydroxy-midazolam, and at 100 ng/mL, the values were 7.5% for midazolam and 11.1% for 1-hydroxy-midazolam. The between-day CV at 1 ng/mL was 9.8% and 13.9% for midazolam and 1-hydroxy-midazolam, respectively. At 10 ng/mL, values were 13.0% and 13.9% for midazolam and 1-hydroxy-midazolam, respectively. At 100 ng/mL, values were 8.2% for midazolam and 10.0% for 1-hydroxy-midazolam.

Pharmacokinetic and Statistical Analysis

Plasma midazolam concentrations after intravenous and oral dosage were analyzed by model-independent methods.⁴² The slope (beta) of the visually identified terminal log-linear phase of the plasma concentration curve was determined by log-linear regression. This was used to calculate the apparent elimination half-life ($t_{1/2} = (\ln 2)/\beta$). Area under the plasma concentration curve from time 0 until the last detectable concentration was calculated using the linear trapezoidal method. To this we added the residual area extrapolated to infinity (final concentration divided by beta), yielding the total area under the curve (AUC). After intravenous midazolam, standard methods were used to calculate volume of distribution by the area method (V_d) and clearance. Assuming hepatic blood flow to be 21.43 mL/min/kg

Table I Effect of Pomegranate Juice or Grapefruit Juice on 50% Inhibitory Concentrations (IC₅₀) Versus Triazolam Hydroxylation by Human Liver Microsomes In Vitro

Liver Sample	Mean (\pm SE, n = 4) IC ₅₀ Value			
	α -OH-Triazolam		4-OH-Triazolam	
	Without Preincubation	With Preincubation	Without Preincubation	With Preincubation
Pomegranate juice				
1	0.78	1.11	0.78	1.02
2	0.34	0.71	0.31	0.65
3	0.86	1.20	0.76	1.09
4	0.45	0.88	0.44	0.83
Mean (\pm SE)	0.61 (\pm 0.13)	0.97 (\pm 0.11)	0.57 (\pm 0.12)	0.90 (\pm 0.10)
Student's <i>t</i>	16.43 ($P < .001$)		9.91 ($P < .005$)	
Aggregate*	0.57	0.96	0.55	0.87
Grapefruit juice				
1	0.50	0.43	0.50	0.42
2	0.55	0.47	0.49	0.43
3	0.62	0.36	0.59	0.34
4	0.53	0.37	0.42	0.19
Mean (\pm SE)	0.55 (\pm 0.03)	0.41 (\pm 0.03)	0.50 (\pm 0.04)	0.35 (\pm 0.05)
Student's <i>t</i>	3.38 ($P < .05$)		3.22 ($P < .05$)	
Aggregate*	0.55	0.41	0.51	0.35

IC₅₀ values are expressed in percent (volume/volume) of fruit beverage in incubation mixture. Asterisk (*) indicates aggregate IC₅₀ values based on analysis of mean fractional inhibition at each concentration (see Figure 1).

(1500 mL/min in a 70-kg person), the extraction ratio (ER) and apparent bioavailability across the hepatic extraction site (F_H) were also calculated. After oral midazolam, calculated parameters included apparent oral clearance, net measured bioavailability (F), and apparent bioavailability across the enteric extraction site (F_C).⁴²

Kinetic parameters after intravenous midazolam were also calculated based on nonlinear regression analysis, in which a linear sum of 2 exponential terms (consistent with a 2-compartment model) was fitted to data points.⁴² This analysis yielded pharmacokinetic parameters nearly identical to those derived from the model-independent analysis.

Statistical methods included Student's paired *t* test or analysis of variance (ANOVA) for repeated measures. Following the ANOVA, individual treatments were compared to each other using the Student-Newman-Keuls test.

RESULTS

In Vitro Studies

Both pomegranate juice and grapefruit juice were in vitro inhibitors of triazolam hydroxylation, the index reaction used to profile CYP3A activity^{39,40,43} (Table I,

Figure 1). For α -OH-triazolam formation, the mean (\pm SE, n = 4) IC₅₀ value for pomegranate juice (without preincubation of juice with microsomal protein) was 0.61(\pm 0.01)%. Preincubation of microsomes with pomegranate juice significantly increased the IC₅₀ to 0.97(\pm 0.11)%, indicating a reduction of inhibitory potency due to preincubation. For grapefruit juice, the IC₅₀ without preincubation was 0.55(\pm 0.03)%; after preincubation, this was significantly reduced to 0.41(\pm 0.03)%, indicating increased inhibitory potency. Findings for 4-OH-triazolam formation were very similar (Table I). The mean IC₅₀ values calculated from analysis of each liver sample individually were nearly identical to the IC₅₀ values determined using aggregate data points at each inhibitor concentration (Table I, Figure 1).

The known CYP3A inhibitor ketoconazole^{39,40,42-44} (0.1 μ M) reduced α -OH-triazolam formation rate to 23.3(\pm 1.1)% of control (mean \pm SE) without preincubation. The value was 42.6(\pm 4.8)% of control after preincubation, indicating a reduction of the potency of ketoconazole inhibition due to preincubation.

Clinical Studies

Fifteen healthy male volunteers (mean age, 35 \pm 9 years; mean weight, 83 \pm 11 kg) enrolled in the trial.

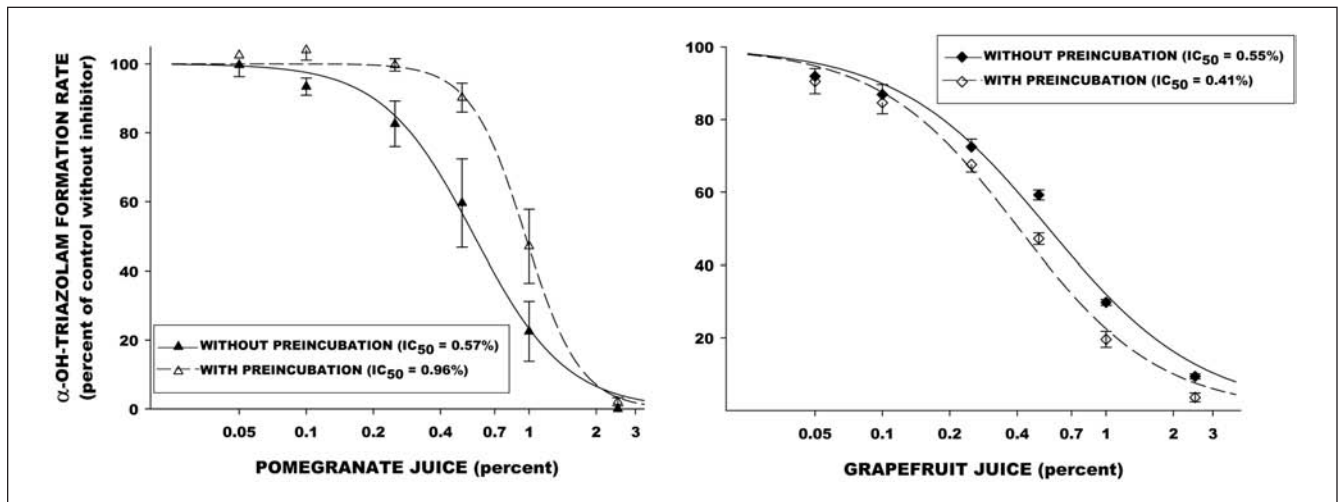


Figure 1. Mean (\pm SE, $n = 4$) triazolam hydroxylation activities (expressed as percent of inhibitor-free control) in relation to co-added concentration of pomegranate juice (left) or grapefruit juice (right). IC_{50} represents the 50% inhibitory concentration determined by non-linear regression analysis of aggregate data. Studies were done both without and with preincubation of juice with microsomal protein. See Table I for data analysis.

The subjects' racial/ethnic backgrounds were: 10 Caucasian, 3 Black, 1 Hispanic, and 1 Asian.

One subject discontinued participation after 2 trials for administrative reasons; data for that subject were not included in the analysis. A second subject completed all 5 trials, but plasma levels from 1 of the trials could not be interpreted due to outlying high values, possibly related to a sample collection artifact. Although none of the data from this subject are incorporated in the statistical analysis, the conclusions of the study were not altered when the data from this subject's 4 interpretable trials were included. The final data analysis is based on 13 subjects who completed all 5 trials. The demographic characteristics of the 13 completers were as follows: mean age, 35 ± 8 years; mean weight, 81 ± 10 kg. The racial/ethnic distribution was: 9 Caucasian, 2 Black, 1 Hispanic, and 1 Asian. There were no adverse events among the subjects.

The pharmacokinetic profile of intravenous midazolam was not significantly different between the control trial (intravenous midazolam with water) and the pomegranate juice coadministration trial (Table II, Figure 2). There also was no significant difference between the 2 trials in total AUC for 1-hydroxymidazolam (3.8 ± 0.3 vs 4.5 ± 0.4 ng/mL \times h).

ANOVA for repeated measures indicated a highly significant difference among the 3 oral midazolam dosage trials in midazolam C_{max} , total AUC, and apparent oral clearance (Table II, Figure 3). The Student-Newman-Keuls test showed that the control and

pomegranate juice trials did not differ from each other, while both were significantly different from the grapefruit juice trial. Coadministration of oral midazolam with grapefruit juice significantly increased C_{max} and AUC and significantly reduced oral clearance (Figure 4). Thus, grapefruit juice, but not pomegranate juice, impaired clearance and increased AUC of orally administered midazolam.

DISCUSSION

This study confirmed previous work^{35,36} demonstrating that pomegranate juice is an inhibitor of CYP3A activity in vitro. Currently, there is no hypothesis regarding which component in the juice might be responsible for its inhibitory effects. Pomegranate juice is not known to contain furanocoumarins such as 6,7'-dihydroxybergamottin, the components in grapefruit juice most likely to account for clinical interactions with CYP3A substrates.^{16,20-30} However, pomegranate juice does contain components which have been shown to inhibit CYP3A, such as quercetin, kaempferol, and gallic acid.^{43,45-48} However, it is not known whether these compounds are found in high enough concentrations to account for the observed inhibition. Furthermore, if quercetin and kaempferol are present as glycoside conjugates, they are less likely to produce CYP inhibition. Ellagic acid, an antioxidant found in large quantities in pomegranate juice, has been shown to inhibit CYP2A2, 3A1, 2C11, 2B1, 2B2, and 2C6 in rat liver microsomes,⁴⁹ and caffeic acid, another

Table II Kinetic Variables for Intravenous and Oral Midazolam When Coadministered With Water, Pomegranate Juice, or Grapefruit Juice

	Mean (\pm SE, n = 13)			Statistical Test
	With Water	With PJ	With GFJ	
Intravenous midazolam				
Volume of distribution				
liters	142 (\pm 12)	134 (\pm 9)		$t = 1.23$ (ns)
liters/kg	1.74 (\pm 0.13)	1.64 (\pm 0.09)		$t = 1.17$ (ns)
Half-life, h	3.67 (\pm 0.29)	3.51 (\pm 0.30)		$t = 0.57$ (ns)
Total AUC, ng/mL \times h	78.7 (\pm 7.4)	83.7 (\pm 8.6)		$t = 1.54$ (ns)
Clearance				
mL/min	463 (\pm 37)	437 (\pm 33)		$t = 1.27$ (ns)
mL/min/kg	5.74 (\pm 0.47)	5.43 (\pm 0.44)		$t = 1.15$ (ns)
Oral midazolam				
C_{max} , ng/mL	28.0 (\pm 2.9)	22.5 (\pm 2.6)	38.2 (\pm 4.3)	$F = 18.3$ ($P < .001$)*
Half-life, h	3.12 (\pm 0.21)	3.37 (\pm 0.31)	3.58 (\pm 0.22)	$F = 1.43$ (ns)
Total AUC, ng/mL \times h	96.5 (\pm 15.7)	84.2 (\pm 12.4)	147.8 (\pm 30.1)	$F = 9.60$ ($P < .001$)*
Clearance				
mL/min	1267 (\pm 133)	1461 (\pm 136)	910 (\pm 117)	$F = 10.12$ ($P < .001$)*
mL/min/kg	16.0 (\pm 2.0)	17.7 (\pm 1.9)	11.1 (\pm 1.4)	$F = 10.17$ ($P < .001$)*
F	0.40 (\pm 0.04)	0.34 (\pm 0.03)		$t = 1.92$ (ns)
F_H	0.73 (\pm 0.02)	0.75 (\pm 0.02)		$t = 1.43$ (ns)
F_G	0.56 (\pm 0.06)	0.46 (\pm 0.04)		$t = 1.78$ (ns)

PJ, pomegranate juice; GFJ, grapefruit juice; AUC, area under the plasma concentration curve; F, observed net oral bioavailability; F_H , calculated bioavailability across the hepatic extraction site; F_G , calculated bioavailability across the enteric extraction site.

*Results of Student-Newman-Keuls test (following ANOVA): water and PJ conditions not significantly different from each other; GFJ condition significantly different ($P < .05$) from water and PJ conditions.

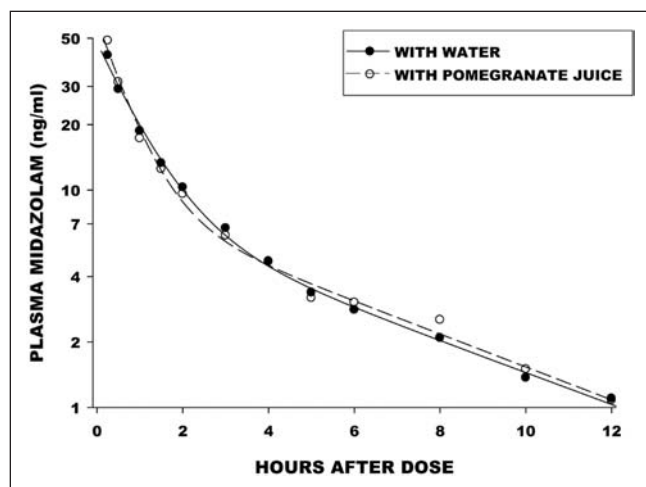


Figure 2. Mean plasma midazolam concentrations (n = 13) after 2 mg intravenous (IV) midazolam preceded by water or by pomegranate juice. Lines represent functions formed by a linear sum of 2 exponential terms, determined by nonlinear regression.

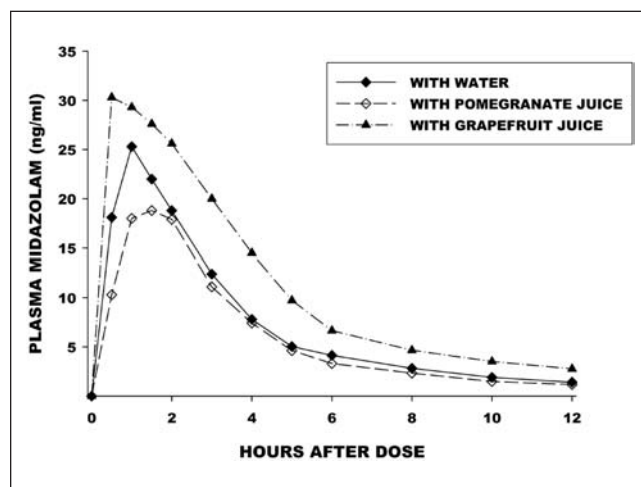


Figure 3. Mean plasma midazolam concentrations (n = 13) after 6 mg oral midazolam preceded by water, pomegranate juice, or grapefruit juice.

antioxidant, was shown to slightly increase rat UDP-glucuronosyltransferase expression in vivo but had no effect on CYPs 1A1, 2B1/2, 2E1, glutathione

S-transferase, and quinone reductase.⁵⁰ However, the effects of ellagic and caffeic acids on the human enzymes are still not known.

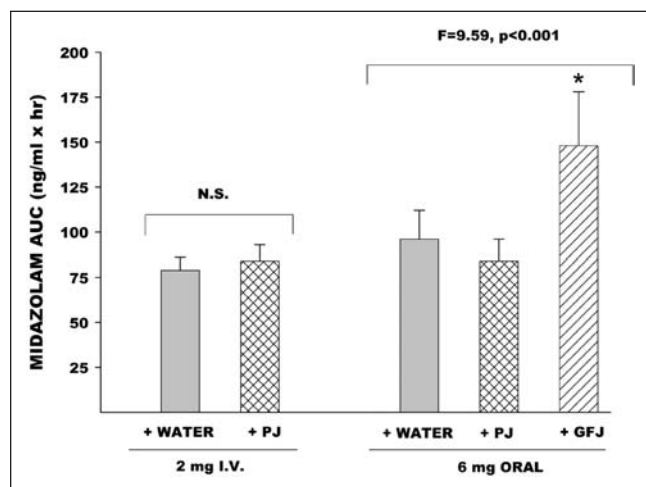


Figure 4. Mean (\pm SE, $n = 13$) total area under the midazolam plasma concentration curve (AUC) in the 5 treatment conditions. See Table II for statistical analysis. Asterisk (*) indicates a significant difference ($P < .05$) between the grapefruit juice (GFJ) condition and both the water and the pomegranate juice (PJ) conditions based on the Student-Newman-Keuls test.

The *in vitro* studies demonstrated that the mode of CYP3A inhibition by pomegranate juice differed from that of grapefruit juice. The IC_{50} for grapefruit juice was reduced with preincubation, whereas the IC_{50} of pomegranate juice was increased with preincubation. Preincubation of an inhibitor with the enzyme can increase the apparent inhibitory potency if the inhibitor binds irreversibly to the enzyme and deactivates it.⁵¹ This is known to be the case for grapefruit juice, which contains compounds that are mechanism-based inhibitors of CYP3A. However, in the case of pomegranate juice, the inhibitor is consumed (inactivated) during the preincubation process, resulting in a higher apparent IC_{50} (reduced inhibitory potency) after preincubation.

Midazolam was chosen as the test substrate in the clinical study for several reasons. This drug is metabolized relatively selectively by CYP3A, and it has 1 primary metabolite, 1'-hydroxy-midazolam,⁵²⁻⁵⁴ and it can be administered both orally and intravenously. Therefore, midazolam can be used to profile both hepatic and intestinal CYP3A activity. With intravenous administration of midazolam, primarily hepatic CYP3A activity is monitored since intestinal metabolism is essentially bypassed. When midazolam is administered orally, net clearance depends on a combination of enteric and hepatic metabolism.

The clinical portion of this study shows that consumption of pomegranate juice does not alter the activity of hepatic or intestinal CYP3A in humans.

This contrasts with *in vitro* experiments and *in vivo* studies with rats, which suggested that pomegranate juice was a significant inhibitor of CYP3A.^{35,36} Both the *in vitro* and *in vivo* studies found that the potency of inhibition was similar to that of grapefruit juice. Administration of pomegranate juice to rats significantly inhibited enteric CYP3A metabolism (as measured by epoxidation of carbamazepine) to almost the same extent as grapefruit juice.³⁶ The lack of a clinically significant interaction in humans could be due to the species differences in drug metabolism. For example, midazolam is metabolized to 1'-hydroxy- and 4-hydroxy-midazolam in both species,⁵²⁻⁵⁴ but the metabolism is carried out by the different CYP3A isoforms. In humans, midazolam is metabolized primarily by CYP3A4 and CYP3A5, whereas in rats, the responsible enzymes are CYP3A1, CYP3A2, as well as some members of the CYP2C family.^{55,56} In addition, there are significant differences in the pharmacokinetics of midazolam between humans and rats.⁵⁶ In rats, the metabolism of midazolam is largely dependent on hepatic extraction rather than intestinal clearance.⁵⁶ However, in humans, intestinal metabolism of orally administered midazolam contributes more significantly to the clearance of this drug.⁴² Another difference between humans and rats is the susceptibility of enzymes to inhibition. In the case of inhibition by ketoconazole, rat liver microsomes were less susceptible than those from human liver.⁵⁶

Metabolism or efflux transport of the inhibitor before it reaches its target could also explain the lack of clinically significant CYP3A inhibition by pomegranate juice in humans. In the case of grapefruit juice, inhibition of CYP3A occurs primarily in the intestine, and hepatic metabolism is relatively unaffected.³¹⁻³⁴ There is some evidence, however, that consumption of concentrated grapefruit juice 2 or 3 times a day could prolong the half-life of triazolam⁵⁷ and midazolam,⁵⁸ suggesting an effect on hepatic CYP3A with exposure to large quantities of grapefruit juice.

The discrepancy between the rodent study³⁶ and the clinical trial of pomegranate juice might also be explained by a difference in the administered dose of pomegranate juice. The dose in our study was 8 oz (approximately 227 mL), and in the rat study,³⁶ the animals received 2 mL of juice. Assuming that the average rat weighs 300 g and a human weighs 70 kg, the corresponding quantity of pomegranate juice for the human study would be 467 mL, approximately twice the dose used in our clinical study. However, another clinical study reported no interaction between pomegranate juice and simvastatin (also a CYP3A substrate) even with a high dose of 900 mL per day for 3 days.³⁸

This study confirms previous clinical reports^{16,31,59} showing that grapefruit juice significantly increases the AUC of orally administered midazolam. A previous study with midazolam¹⁶ found that grapefruit juice increased the AUC by a factor of 1.65, similar to the 1.53-fold increase in this study. In conclusion, pomegranate juice does not alter the clearance of oral or intravenous midazolam, suggesting that there is no clinically detectable inhibition of CYP3A activity. Furthermore, in vitro and animal studies of CYP3A inhibition by nutrients and natural substances cannot be assumed to be clinically relevant until demonstrated in humans.

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