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

Dora Farkas, Lauren E. Oleson, Yanli Zhao, Jerold S. Harmatz, Miguel A. Zinny, Michael H. Court, and David J. Greenblatt

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  $\alpha$ -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.<sup>1</sup> 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.<sup>1-4</sup> 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,<sup>5</sup> suppresses prostate<sup>6</sup> and breast cancers,<sup>7</sup> and it might even augment the effect of HIV treatment regimens.<sup>8</sup>

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.<sup>9,10</sup> 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.<sup>11-15</sup> 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.<sup>16-19</sup> One compound that appears to contribute to the observed inhibition is 6,7'-dihydroxybergamottin (DHB),<sup>16,20-30</sup> 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.<sup>16,20-30</sup> Clinical studies also demonstrate that grapefruit juice inhibits enteric CYP3A more than hepatic CYP3A.<sup>31-34</sup> Therefore, the consumption of grapefruit juice primarily affects the clearance of orally administered drugs which normally undergo significant metabolism by enteric CYP3A.<sup>11</sup> 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.<sup>31-34</sup>

Recent reports suggest that other fruit juices can also inhibit CYP 3A activity. Hidaka et al<sup>35</sup> 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.<sup>36</sup> 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,<sup>37</sup> but the number of adequately controlled pharmacokinetic studies is small.<sup>38</sup>

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.<sup>39</sup> 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 µmol/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 × 3.9 mm, containing reverse-phase C18 Novapak (Waters Associates, Milford, Mass). Column effluent was monitored by ultraviolet absorbance at 220 nanometers.<sup>39,40</sup> 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 ( $IC_{50}$ ).<sup>40</sup>

### 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  $\mu$ 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.<sup>41</sup> 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  $\mu$ 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.<sup>42</sup> 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 (IC<sub>50</sub>) Versus Triazolam Hydroxylation by Human Liver Microsomes In Vitro

Liver Sample	Mean ( $\pm$ SE, n = 4) IC <sub>50</sub> Value			
	$\alpha$ -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

IC<sub>50</sub> values are expressed in percent (volume/volume) of fruit beverage in incubation mixture. Asterisk (\*) indicates aggregate IC<sub>50</sub> 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 ( $F_C$ ).<sup>42</sup>

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.<sup>42</sup> 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<sup>39,40,43</sup> (Table I,

Figure 1). For  $\alpha$ -OH-triazolam formation, the mean ( $\pm$ SE, n = 4) IC<sub>50</sub> 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 IC<sub>50</sub> to 0.97( $\pm$ 0.11)%, indicating a reduction of inhibitory potency due to preincubation. For grapefruit juice, the IC<sub>50</sub> 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 IC<sub>50</sub> values calculated from analysis of each liver sample individually were nearly identical to the IC<sub>50</sub> values determined using aggregate data points at each inhibitor concentration (Table I, Figure 1).

The known CYP3A inhibitor ketoconazole<sup>39,40,42-44</sup> (0.1  $\mu$ M) reduced  $\alpha$ -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.

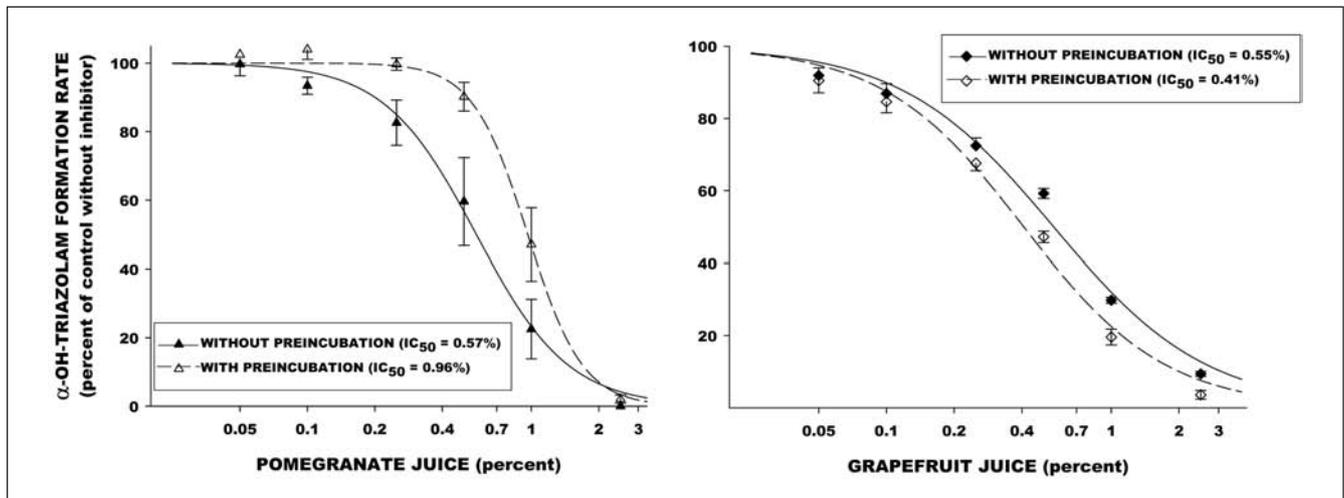


Figure 1. Mean ( $\pm$ SE,  $n = 4$ ) triazolam hydroxylation activities (expressed as percent of inhibitor-free control) in relation to co-added concentration of pomegranate juice (left) or grapefruit juice (right).  $IC_{50}$  represents the 50% inhibitory concentration determined by non-linear regression 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,  $35 \pm 8$  years; mean weight,  $81 \pm 10$  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 ( $3.8 \pm 0.3$  vs  $4.5 \pm 0.4$  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<sup>35,36</sup> 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.<sup>16,20-30</sup> However, pomegranate juice does contain components which have been shown to inhibit CYP3A, such as quercetin, kaempferol, and gallic acid.<sup>43,45-48</sup> 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,<sup>49</sup> 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	
<b>Intravenous midazolam</b>				
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)
<b>Oral midazolam</b>				
$C_{max}$ , 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.

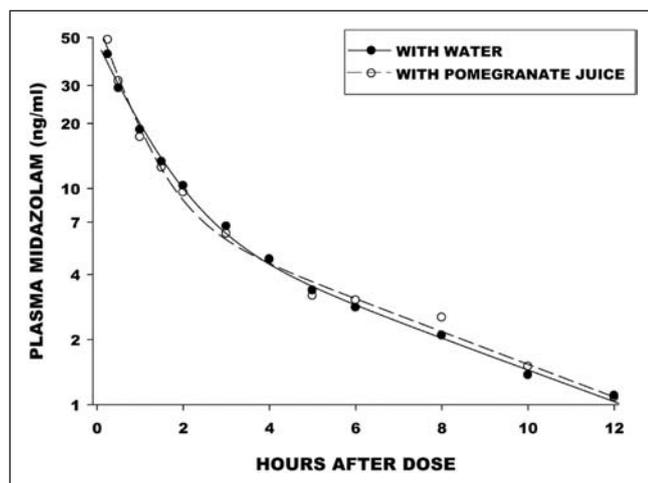


Figure 2. Mean plasma midazolam concentrations ( $n = 13$ ) after 2 mg intravenous (IV) midazolam preceded by water or by pomegranate juice. Lines represent functions formed by a linear sum of 2 exponential terms, determined by nonlinear regression.

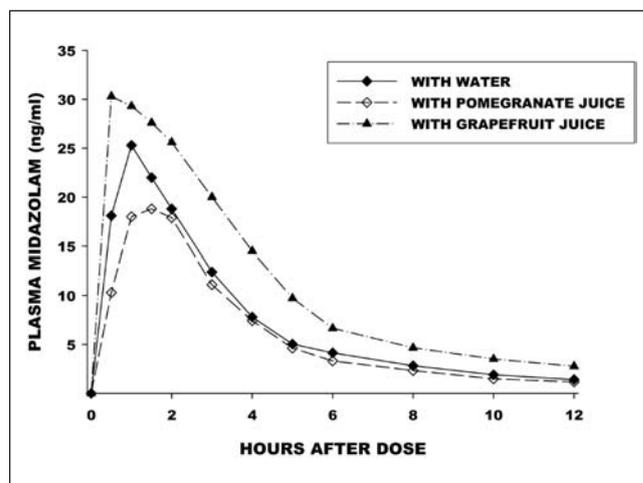


Figure 3. Mean plasma midazolam concentrations ( $n = 13$ ) after 6 mg oral midazolam preceded by water, pomegranate juice, or grapefruit juice.

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.<sup>50</sup> However, the effects of ellagic and caffeic acids on the human enzymes are still not known.

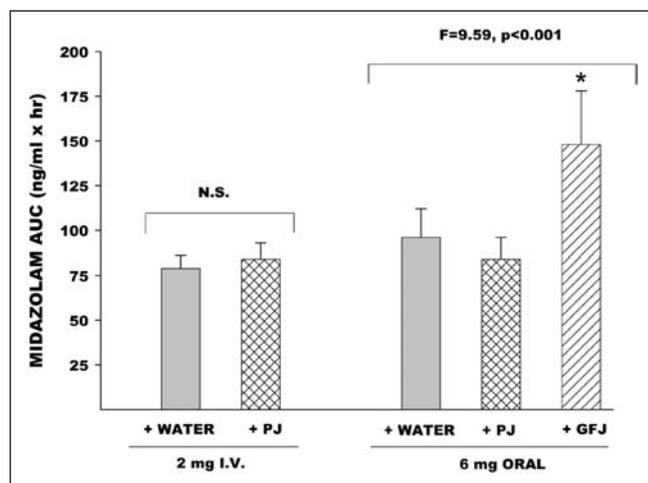


Figure 4. Mean ( $\pm$  SE,  $n = 13$ ) total area under the midazolam plasma concentration curve (AUC) in the 5 treatment conditions. See Table II for statistical analysis. Asterisk (\*) indicates a significant difference ( $P < .05$ ) between the grapefruit juice (GFJ) condition and both the water 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.<sup>51</sup> 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,<sup>52-54</sup> 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.<sup>35,36</sup> 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.<sup>36</sup> 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,<sup>52-54</sup> 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.<sup>55,56</sup> In addition, there are significant differences in the pharmacokinetics of midazolam between humans and rats.<sup>56</sup> In rats, the metabolism of midazolam is largely dependent on hepatic extraction rather than intestinal clearance.<sup>56</sup> However, in humans, intestinal metabolism of orally administered midazolam contributes more significantly to the clearance of this drug.<sup>42</sup> 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.<sup>56</sup>

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.<sup>31-34</sup> There is some evidence, however, that consumption of concentrated grapefruit juice 2 or 3 times a day could prolong the half-life of triazolam<sup>57</sup> and midazolam,<sup>58</sup> suggesting an effect on hepatic CYP3A with exposure to large quantities of grapefruit juice.

The discrepancy between the rodent study<sup>36</sup> 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,<sup>36</sup> 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.<sup>38</sup>

This study confirms previous clinical reports<sup>16,31,59</sup> showing that grapefruit juice significantly increases the AUC of orally administered midazolam. A previous study with midazolam<sup>16</sup> 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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## REFERENCES

1. Lei F, Xing DM, Xiang L, et al. Pharmacokinetic study of ellagic acid in rat after oral administration of pomegranate leaf extract. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003;796:189-194.
2. Perez-Vicente A, Gil-Izquierdo A, Garcia-Viguera C. In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. *J Agric Food Chem*. 2002;50:2308-2312.
3. Seeram NP, Lee R, Heber D. Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (*Punica granatum* L.) juice. *Clin Chim Acta*. 2004;348:63-68.
4. Cerda B, Espin JC, Parra S, Martinez P, Tomas-Barberan FA. The potent in vitro antioxidant ellagitannins from pomegranate juice are metabolised into bioavailable but poor antioxidant hydroxy-6H-dibenzopyran-6-one derivatives by the colonic microflora of healthy humans. *Eur J Nutr*. 2004;43:205-220.
5. Aviram M, Dornfeld L, Kaplan M, et al. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. *Drugs Exp Clin Res*. 2002;28:49-62.
6. Lansky EP, Jiang W, Mo H, et al. Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions. *Invest New Drugs*. 2005;23:11-20.
7. Kim ND, Mehta R, Yu W, et al. Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. *Breast Cancer Res Treat*. 2002;71:203-217.
8. Neurath AR, Strick N, Li YY, Debnath AK. *Punica granatum* (pomegranate) juice provides an HIV-1 entry inhibitor and candidate topical microbicide. *BMC Infect Dis*. 2004;4:41.
9. Bailey DG, Spence JD, Edgar B, Bayliff CD, Arnold JM. Ethanol enhances the hemodynamic effects of felodipine. *Clin Invest Med*. 1989;12:357-362.
10. Bailey DG, Spence JD, Munoz C, Arnold JM. Interaction of citrus juices with felodipine and nifedipine. *Lancet*. 1991;337:268-269.
11. Greenblatt DJ, Patki KC, von Moltke LL, Shader RI. Drug interactions with grapefruit juice: an update. *J Clin Psychopharmacol*. 2001;21:357-359.
12. Bailey DG, Arnold JMO, Spence JD. Grapefruit juice–drug interactions. *Br J Clin Pharmacol*. 1998;46:101-110.
13. Kane GC, Lipsky JJ. Drug–grapefruit juice interactions. *Mayo Clinic Proc*. 2000;75:933-942.
14. Ameer B, Weintraub RA. Drug interactions with grapefruit juice. *Clin Pharmacokinet*. 1997;33:103-121.
15. Mertens-Talcott SU, Zadezensky I, De Castro WV, Derendorf H, Butterweck V. Grapefruit–drug interactions: can interactions with drugs be avoided? *J Clin Pharmacol*. 2006;46:1390-1416.
16. Greenblatt DJ, von Moltke LL, Harmatz JS, et al. Time course of recovery of cytochrome P450 3A function after single doses of grapefruit juice. *Clin Pharmacol Ther*. 2003;74:121-129.
17. Lilja JJ, Kivistö KT, Neuvonen PJ. Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin. *Clin Pharmacol Ther*. 2000;68:384-390.
18. Takanaga H, Ohnishi A, Matsuo H, et al. Pharmacokinetic analysis of felodipine–grapefruit juice interaction based on an irreversible enzyme inhibition model. *Br J Clin Pharmacol*. 2000;49:49-58.
19. Takanaga H, Ohnishi A, Murakami H, et al. Relationship between time after intake of grapefruit juice and the effect on pharmacokinetics and pharmacodynamics of nisoldipine in healthy subjects. *Clin Pharmacol Ther*. 2000;67:201-214.
20. Edwards DJ, Fitzsimmons ME, Schuetz EG, et al. 6,7'-Dihydroxybergamottin in grapefruit juice and Seville orange juice: effects on cyclosporine disposition, enterocyte CYP3A4, and P-glycoprotein. *Clin Pharmacol Ther*. 1999;65:237-244.
21. Paine MF, Criss AB, Watkins PB. Two major grapefruit juice components differ in intestinal CYP3A4 inhibition kinetic and binding properties. *Drug Metab Dispos*. 2004;32:1146-1153.
22. Paine MF, Criss AB, Watkins PB. Two major grapefruit juice components differ in time to onset of intestinal CYP3A4 inhibition. *J Pharmacol Exp Ther*. 2005;312:1151-1160.
23. Guo LQ, Fukuda K, Ohta T, Yamazoe Y. Role of furanocoumarin derivatives on grapefruit juice–mediated inhibition of human CYP3A activity. *Drug Metab Dispos*. 2000;28:766-771.
24. Fukuda K, Ohta T, Oshima Y, Ohashi N, Yoshikawa M, Yamazoe Y. Specific CYP3A4 inhibitors in grapefruit juice: furocoumarin dimers as components of drug interaction. *Pharmacogenetics*. 1997;7:391-396.
25. Edwards DJ, Bellevue FH III, Woster PM. Identification of 6,7'-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. *Drug Metab Dispos*. 1996;24:1287-1290.
26. Girennavar B, Poulouse SM, Jayaprakasha GK, Bhat NG, Patil BS. Furocoumarins from grapefruit juice and their effect on human CYP 3A4 and CYP 1B1 isoenzymes. *Bioorg Med Chem*. 2006;14:2606-2612.
27. Guo LQ, Taniguchi M, Xiao YQ, Baba K, Ohta T, Yamazoe Y. Inhibitory effect of natural furanocoumarins on human microsomal cytochrome P450 3A activity. *Jpn J Pharmacol*. 2000;82:122-129.
28. Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME, et al. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents: decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. *Drug Metab Dispos*. 1997;25:1228-1233.
29. Paine MF, Widmer WW, Hart HL, et al. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice–felodipine interaction. *Am J Clin Nutr*. 2006;83:1097-1105.

30. Widmer W, Haun C. Variation in furanocoumarin content and new furanocoumarin dimers in commercial grapefruit (*Citrus paradisi* Macf.) juices. *J Food Sci.* 2005;70:c307-c312.
31. Kupferschmidt HHT, Ha HR, Ziegler WH, Meier PJ, Krähenbühl S. Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther.* 1995;58:20-28.
32. Ducharme MP, Warbasse LH, Edwards DJ. Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. *Clin Pharmacol Ther.* 1995;57:485-491.
33. Lundahl J, Regardh CG, Edgar B, Johnsson G. Effects of grapefruit juice ingestion: pharmacokinetics and haemodynamics of intravenously and orally administered felodipine in healthy men. *Eur J Clin Pharmacol.* 1997;52:139-145.
34. Uno T, Ohkubo T, Sugawara K, Higashiyama A, Motomura S, Ishizaki T. Effects of grapefruit juice on the stereoselective disposition of nifedipine in humans: evidence for dominant presystemic elimination at the gut site. *Eur J Clin Pharmacol.* 2000;56:643-649.
35. Hidaka M, Fujita K, Ogikubo T, et al. Potent inhibition by star fruit of human cytochrome P450 3A (CYP3A) activity. *Drug Metab Dispos.* 2004;32:581-583.
36. Hidaka M, Okumura M, Fujita K, et al. Effects of pomegranate juice on human cytochrome P450 3A (CYP3A) and carbamazepine pharmacokinetics in rats. *Drug Metab Dispos.* 2005;33:644-648.
37. Sorokin AV, Duncan B, Panetta R, Thompson PD. Rhabdomyolysis associated with pomegranate juice consumption. *Am J Cardiol.* 2006;98:705-706.
38. Yeo C, Shon J, Liu K, Lee D, Yoon Y, Shin J. The effects of pomegranate juice on pharmacokinetics of simvastatin in healthy Korean subjects [abstract]. *Clin Pharmacol Ther.* 2006;79:P23.
39. von Moltke LL, Greenblatt DJ, Harmatz JS, et al. Triazolam biotransformation by human liver microsomes in vitro: effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole. *J Pharmacol Exp Ther.* 1996;276:370-379.
40. von Moltke LL, Greenblatt DJ, Grassi JM, et al. Protease inhibitors as inhibitors of human cytochromes P450: high risk associated with ritonavir. *J Clin Pharmacol.* 1998;38:106-111.
41. Culm-Merdek KE, von Moltke LL, Gan L, et al. Effect of extended exposure to grapefruit juice on cytochrome P450 3A activity in humans: comparison with ritonavir. *Clin Pharm Ther.* 2006;79:243-254.
42. Tsunoda SM, Velez RL, von Moltke LL, Greenblatt DJ. Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an in vivo probe: effect of ketoconazole. *Clin Pharmacol Ther.* 1999;66:461-471.
43. von Moltke LL, Weemhoff JL, Bedir E, et al. Inhibition of human cytochromes P450 by components of *Ginkgo biloba*. *J Pharm Pharmacol.* 2004;56:1039-1044.
44. Venkatakrishnan K, von Moltke LL, Greenblatt DJ. Effects of the antifungal agents on oxidative drug metabolism in humans: clinical relevance. *Clin Pharmacokinet.* 2000;38:111-180.
45. He N, Edeki T. The inhibitory effects of herbal components on CYP2C9 and CYP3A4 catalytic activities in human liver microsomes. *Am J Ther.* 2004;11:206-212.
46. Patel J, Buddha B, Dey S, Pal D, Mitra AK. In vitro interaction of the HIV protease inhibitor ritonavir with herbal constituents: changes in P-gp and CYP3A4 activity. *Am J Ther.* 2004;11:262-277.
47. Tsukamoto S, Tomise K, Aburatani M, et al. Isolation of cytochrome P450 inhibitors from strawberry fruit, *Fragaria ananassa*. *J Nat Prod.* 2004;67:1839-1841.
48. Stupans L, Tan HW, Kirlich A, Tuck K, Hayball P, Murray M. Inhibition of CYP3A-mediated oxidation in human hepatic microsomes by the dietary derived complex phenol, gallic acid. *J Pharm Pharmacol.* 2002;54:269-275.
49. Zhang Z, Hamilton SM, Stewart C, Strother A, Teel RW. Inhibition of liver microsomal cytochrome P450 activity and metabolism of the tobacco-specific nitrosamine NNK by capsaicin and ellagic acid. *Anticancer Res.* 1993;13:2341-2346.
50. Debersac P, Vernevaut MF, Amiot MJ, Suschetet M, Siess MH. Effects of a water-soluble extract of rosemary and its purified component rosmarinic acid on xenobiotic-metabolizing enzymes in rat liver. *Food Chem Toxicol.* 2001;39:109-117.
51. Silverman R. Mechanism-based enzyme inactivators. *Methods Enzymol.* 1995;249:240-283.
52. Kronbach T, Mathys D, Umeno M, Gonzalez FJ, Meyer UA. Oxidation of midazolam and triazolam by human liver cytochrome P450 IIIA4. *Mol Pharmacol.* 1989;36:89-96.
53. von Moltke LL, Greenblatt DJ, Schmider J, et al. Midazolam hydroxylation by human liver microsomes in vitro: inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents. *J Clin Pharmacol.* 1996;36:783-791.
54. Perloff MD, von Moltke LL, Court MH, Kotegawa T, Shader RI, Greenblatt DJ. Midazolam and triazolam biotransformation in mouse and human liver microsomes: relative contribution of CYP3A and CYP2C isoforms. *J Pharmacol Exp Ther.* 2000;292:618-628.
55. Ghosal A, Satoh H, Thomas P, Bush E, Moore D. Inhibition and kinetics of cytochrome P4503A activity in microsomes from rat, human, and cDNA-expressed human cytochrome P450. *Drug Metab Dispos.* 1996;24:940-947.
56. Kotegawa T, Laurijssens BE, von Moltke LL, et al. In vitro, pharmacokinetic, and pharmacodynamic interactions of ketoconazole and midazolam in the rat. *J Pharmacol Exp Ther.* 2002;302:1228-1237.
57. Lilja JJ, Kivistö KT, Backman JT, Neuvonen PJ. Effect of grapefruit juice dose on grapefruit juice-triazolam interaction: repeated consumption prolongs triazolam half-life. *Eur J Clin Pharmacol.* 2000;56:411-415.
58. Veronese ML, Gillen LP, Burke JP, et al. Exposure-dependent inhibition of intestinal and hepatic CYP3A4 in vivo by grapefruit juice. *J Clin Pharmacol.* 2003;43:831-839.
59. Andersen V, Pedersen N, Larsen NE, Sonne J, Larsen S. Intestinal first pass metabolism of midazolam in liver cirrhosis: effect of grapefruit juice. *Br J Clin Pharmacol.* 2002;54:120-124.