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Effect of Low Furanocoumarin Hybrid Grapefruit Juice Consumption on Midazolam Pharmacokinetics

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Abstract

The objectives of this study were to investigate the effect of grapefruit juice low in furanocoumarins on CYP3A activity and to summarize previous findings of enzyme inhibition measured by the metabolism of midazolam after intake of grapefruit juice. Twelve healthy volunteers participated in a prospective, randomized, double-blinded, three-way crossover clinical study to determine the effect of regular grapefruit juice (RGJ) and a novel, low furanocoumarin hybrid grapefruit juice (HGJ) on the metabolism of oral midazolam, used as a probe for *in vivo* CYP3A activity, compared with water as a control. The RGJ was 100% hand-squeezed Hudson grapefruit juice, and the HGJ contained low amounts of furanocoumarin constituents. The point estimates (90% confidence intervals) for the RGJ/water midazolam AUC geometric mean ratio was 122% (107 – 140). The point estimate for the HGJ/water midazolam AUC ratio was within the 80%-125% bioequivalence range, indicating an absence of interaction. This finding also prompted a systematic review of available evidence on the pharmacokinetic alteration of midazolam by grapefruit juice. While most studies demonstrated alteration in midazolam pharmacokinetics supporting inhibition of CYP3A activity as a likely mechanism, the cohorts included in these studies and the extent of the pharmacokinetic interaction varied widely. The current study indicated grapefruit juice-drug interaction varies substantially based on patient

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Conflicts of Interests:

Dr. Gmitter is an inventor on US patent application #US20140283239 P1 and USPP26177 P3 of hybrid grapefruit 914. Dr. Gmitter did not analyze any of the data nor did he recruit, consent or interact with any of the participants in the study. None of the other authors have any competing interests.

characteristics and/or grapefruit juice product-related factors, including the amount of furanocoumarin constituents present in the juice.

Keywords

grapefruit juice; drug-food interaction; CYP3A; midazolam

Introduction

Over the past quarter of a century, grapefruit juice (GFJ) has increasingly been cautioned because of potential risk for drug interactions. In 1989, the potential for a drug interaction with GFJ was discovered accidentally, when GFJ was used to mask the taste of ethanol in a felodipine drug-interaction study.¹ Subsequent work has clearly established that grapefruit juice inhibits the activity of human cytochrome P450 (CYP) 3A enzymes and alters the pharmacokinetics (PK) of many medications, especially those with intrinsically low oral bioavailability due to presystemic metabolism mediated by the CYP3A enzyme system.² According to a recent report, grapefruit or GFJ interacts or is predicted to interact with more than 85 medications, of which 43 have interactions leading to potentially serious adverse drug reactions.³ Drugs with previously reported serious adverse events linked to grapefruit-drug interaction include amiodarone, quinine, verapamil, atorvastatin, simvastatin, tacrolimus, colchicine, and ethinylestradiol.³ CYP enzymes in the families of CYP 1, 2, and 3 are responsible for metabolism of 70-80% of all drugs; CYP3A4 metabolizes over 30% of clinically used drugs that undergo biotransformation by CYP isoforms.⁴

Midazolam is an ultra-short acting benzodiazepine derivative commonly used as an *in vivo* probe to measure human CYP3A activity.^{5, 6} After oral administration, midazolam undergoes extensive presystemic extraction by hepatic and enteric CYP3A, and its oral bioavailability is approximately 30%.⁶ The major primary metabolite of midazolam is 1'-hydroxymidazolam generated almost exclusively by CYP3A.⁷ Midazolam was previously used to investigate the effect of GFJ on its metabolism; increased drowsiness and impaired psychomotor measurements have been reported with concomitant intake of GFJ in previous studies.⁸ While increased midazolam exposure has been reported by measurements of area under the concentration-time curve (AUC) and peak plasma concentration ($C_{\max}$) after GFJ intake,⁸ the extent of changes in the AUC and $C_{\max}$ among published clinical trials has been inconsistent. GFJ inhibits intestinal CYP3A, does not impact the PK of intravenously administered drugs, and the plasma half-life of orally ingested drugs are generally not affected by GFJ.⁹

GFJ contains various phytochemicals such as flavonoids and furanocoumarins.⁹ Naringin is the most abundant flavonoid and was originally assumed to be the constituent responsible for the CYP3A inhibition.⁹ However, studies failed to document alternations in the PK of CYP3A substrates, and thus naringin is no longer considered a major constituent accountable for GFJ drug interactions.⁹⁻¹¹ Furanocoumarins are structurally distinct compounds; bergamottin and 6',7'-dihydroxybergamottin (DHB) are the main furanocoumarin components.^{9, 12} These two furanocoumarins are the most extensively

studied for their capability of mediating drug interaction and have been shown to inhibit *in vitro* activity of CYP3A.^{9, 12}

The effect of GFJ from a cultivar specifically bred to contain only limited amounts of furanocoumarins on CYP3A-mediated metabolism has not been investigated. Therefore, the primary objective of this study was to determine how the effects of low-furanocoumarin hybrid grapefruit juice (HGJ) and regular grapefruit juice (RGJ) on midazolam PK differ from those of water as a control. We also systematically reviewed and summarized the clinical evidence regarding the GFJ-midazolam interaction.

Methods

Study Design and Participants

The study protocol was approved by the Institutional Review Board at the University of Florida, and all participants provided voluntary, written informed consent. A prospective, randomized, double-blinded, 3-way crossover design was used to compare three treatments among healthy adult volunteers. Exclusion criteria included age <18 years old, pregnancy or breast feeding, current smoking, diagnosis of a chronic condition or use of chronic medications except oral contraceptives. During three separate study sessions separated by at least 2 weeks, participants received one of three randomly assigned treatments: 200 mL of either water, Hudson hand-squeezed 100% RGJ, or HGJ daily for 3 consecutive days, administered in the morning. On the 3rd day of each study session, participants were admitted to the Clinical Research Center in a fasting state. A venous access catheter was inserted, and a 5-mg dose of oral midazolam syrup (Roxane Laboratories Inc, Columbus, OH) was administered immediately following the third dose of randomized treatment. Blood samples were collected prior to and at 0.5, 1, 2, 3, 4, 6, and 9 hours after midazolam administration. These samples were kept on ice, centrifuged within 30 min of collection, and stored at -80°C until analysis. All participants abstained from eating grapefruit, other citrus fruits, and any citrus containing foods or drinks for 1 week prior to each study session. Participants were asked to abstain from alcoholic beverages (24 hours), herbal containing supplements/teas/beverages, and over-the-counter medications (48 hours) prior to each study sessions. The subjects were also asked to maintain their usual intake of caffeine. The study is registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02117869) (NCT02117869).

Analytical Methods

Midazolam and 1'-Hydroxymidazolam—Plasma concentrations of midazolam and 1'-hydroxymidazolam were quantified using HPLC-MS/MS on a system consisting of a Surveyor HPLC autosampler, Surveyor MS quaternary pump, and TSQ Quantum Discovery triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA). After liquid-liquid extraction, midazolam and metabolite concentrations were measured simultaneously with electrospray ionization in the positive mode as described previously.¹³ Midazolam, midazolam d4, 1'-hydroxymidazolam, and 1'-hydroxymidazolam-d4 were purchased from Cerilliant (Round Rock, TX). The lower limit of quantification for both midazolam and 1'-hydroxymidazolam was 0.2 ng/mL, and the accuracy and precision were within 7%.

Grapefruit Juice—Juice samples were prepared by hand squeezing fruit from ‘Hudson’ grapefruit, and ‘UF914,’ a triploid hybrid arising from the cross of diploid low-acid pummelo with tetraploid ‘Ruby Red’ (UF914 U.S. patent number PP26,177); fruits from both cultivars were harvested from adjacent fields located at the University of Florida Institute of Food and Agricultural Sciences in Central Florida. Analyses were conducted to confirm low amounts of furanocoumarins, using a Perkin Elmer 200 HPLC system (Perkin Elmer, Waltham, MA). Further characterization was obtained with an LCQ Advantage LC-MS system (ThermoFinnigan, San Jose, CA). The lower limit of quantification was 0.5 mg/L. The detailed methodology is described previously.¹⁴

Pharmacokinetics

Pharmacokinetic parameters ($C_{\max}$ and AUC) were obtained from noncompartmental analyses using Kinetica 5.0 (Thermo Fisher Scientific, Waltham, MA). AUC from time 0 until the last concentration measurement (AUC_{last}) was calculated using the linear-log trapezoidal method. Residual area extrapolated to infinity (final concentration divided by beta) was added to calculate the total area under the concentration-time curve (AUC_{inf}). Metabolic ratio was determined, based on the metabolite-to-midazolam AUC ratio.

Statistical Methods

The pharmacokinetic parameter endpoints AUC_{last} , AUC_{inf} , and $C_{\max}$ for midazolam and 1'-hydroxymidazolam were evaluated using the standard bioequivalence approach in which the absence of a clinically relevant interaction was concluded if the 90% confidence interval (CI) for the geometric mean ratio (GMR) was not contained within the no-effect bounds of 80% to 125%.¹⁵ Based on an intra-subject variation of 17% for midazolam AUC,¹⁶ a sample size of twelve subjects would provide at least 80% power to demonstrate that the 90% CI of the geometric mean ratio would fall within the no-effect range of 80% to 125%. SAS version 9.3 was used for analysis (SAS Institute Inc., Cary, NC). Figures were drawn using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Results

Twelve healthy participants (age 30.8 ± 11.8 years, mean $\pm$ standard deviation) completed all study sessions. Eight (67%) of 12 participants were female, and four (33%) participants were male. The body mass index (BMI) was 24.7 ± 4.9 , and two participants were considered obese (BMI ≥ 30). The participants of this study were ethnically diverse (ten Caucasians [two Hispanics, eight non-Hispanics], one African American, and one Asian). The age of participants ranged from 18 to 55 years. None of the participants reported deviations from the study instructions or withdrew from the study. Mild drowsiness was reported by one participant, but no other adverse drug reactions were observed or reported during any of the sessions.

Measured furanocoumarin concentrations for the RGJ and HGJ products are shown in Table 1. The levels of furanocoumarins were lower in the HGJ than RGJ. Midazolam and metabolite concentration-time curves are depicted in Figure 1. The PK parameters calculated from measured concentrations of midazolam and the metabolite are summarized in Table 2

according to randomized treatment. Compared with water (reference), midazolam AUC was increased by a factor of 1.22 with administration of RGJ; the confidence intervals were not contained within the no-effect bounds (90% CI, 107% - 140%; Figure 2). The midazolam C_{max} was decreased by a factor of 0.92 and the lower end of the confidence limit was <80%, outside of the no-effect bounds. The C_{max} of 1'-hydroxymidazolam was also lower when participants consumed RGJ (65%, Figure 3). The metabolite-to-midazolam AUC metabolic ratios were 0.56 ± 0.23 for water, 0.40 ± 0.12 for RGJ, and 0.46 ± 0.19 for HGJ. The metabolic ratio was lower with RGJ compared with water, whereas the metabolic ratios were relatively similar between water and HGJ (Figure 4). When midazolam was co-administered with HGJ, compared to when midazolam was co-administered with water, the GMR (90% CI) for both midazolam and 1'-hydroxymidazolam AUC were contained within the no-effect boundary of 80% – 125% (Table 2).

Discussion

Although CYP3A inhibition by GFJ has been shown to be both reversible and irreversible, the primary mechanism is via inhibition of intestinal CYP3A, resulting in complete inactivation of the enzyme.^{6, 8, 9} CYP3A enzymes are the most abundantly expressed CYP enzymes in both intestinal enterocytes and the liver, the major sites of human drug metabolism.⁴

The results of this clinical study demonstrated that *in vivo* CYP3A activity measured by the metabolism of midazolam was weakly inhibited by consumption of RGJ. The midazolam AUC was increased when participants ingested RGJ, compared to when participants were randomized to water (GMR 122%). While no significant change in C_{max} of midazolam was observed, both AUC metabolic ratio and the C_{max} of the major metabolite, 1'-hydroxymidazolam, were decreased by the intake of RGJ, suggesting intestinal CYP3A inhibition. These alterations indicate that RGJ inhibited the *in vivo* activity of CYP3A in study participants. In contrast, the PK of midazolam was not affected by HGJ as the GMR and 90% CIs for midazolam $AUC_{0-\infty}$ were within the 80% to 125% limits when compared with water. This lack of an effect on midazolam PK parameters with the HGJ product is most likely explained by the low amounts of furanocoumarin constituents (Table 1).

The observations with the HGJ intake in this current study align with earlier findings. When furanocoumarins were manually removed from GFJ by absorption resins in a previous study, exposure to the CYP3A substrate felodipine was maintained, demonstrating that furanocoumarins were the constituents responsible, at least in part, for the CYP3A inhibition.¹⁷ Furthermore, the DHB along with a series of dimeric compounds (spiroesters) were identified as principal inhibitory furanocoumarins.¹⁸ An earlier *in vitro* study similarly described different inhibitory potencies of furanocoumarin constituents.¹⁹ While deviation of *in vivo* inhibitory potency from *in vitro* studies is expected due to variability in participants' response and differences in concentrations of each furanocoumarin constituent used among studies, the results of this study indicate that compared with control (water), *in vivo* CYP3A activity as measured by midazolam metabolism was not affected by HGJ consumption. Additional *in vivo* investigations in larger study populations would be

necessary to further understand the differences observed in the *in vitro* and *in vivo* work completed to date.

Results from our study prompted a systematic review of the literature to better understand the published data evaluating the GFJ-midazolam PK interaction. Search terms used for PubMed were: (“citrus paradisi”[MeSH Terms] OR (“citrus”[All Fields] AND “paradisi”[All Fields]) OR “citrus paradisi”[All Fields] OR “grapefruit”[All Fields]) AND (“midazolam”[MeSH Terms] OR “midazolam”[All Fields]). Similarly, Web of Science was searched with the term, TS=(grapefruit AND midazolam), and then the search was further refined by the document type “CLINICAL TRIAL.” The search was conducted by three of the authors independently for all articles indexed to the database by May 28, 2015. Inconsistencies between included articles were systematically resolved based on pre-determined criteria and by consensus. Only full-text peer-reviewed articles written in English were included. *In vitro* studies and studies using CYP3A probes other than midazolam were excluded. The searches yielded 45 articles from PubMed and 17 articles from Web of Science. After screening all articles found by both search engines, 12 full-text original research articles met our criteria.²⁰⁻³¹ Data from these clinical studies are summarized in Supplemental Table S1. The majority of previous clinical studies were conducted primarily in white male subjects. Although not all studies reported the specific type of grapefruit, concentrated frozen grapefruit was more frequently studied as the source of juice. Overall, 9 out of the 12 studies demonstrated significant changes in midazolam PK (Supplemental Table S1); two studies using intravenously-administered midazolam and one study using orally-dosed midazolam did not show statistically significant alteration in the midazolam total exposure after intake of GFJ.

According to the summary of the systematic review (Supplemental Table S1), the extent of the PK alteration observed in our study was smaller than those reported in the previous positive studies. One factor, possibly contributing to this lack of agreement between previous and current findings, may be the difference in study populations. This study was unique because participants were more ethnically diverse with females being more represented than in previous studies. Additionally, a more important factor may be differences among GFJ itself leading to varying degrees of inhibition between studies. It is commonly suspected that different cultivars and/or storage conditions of grapefruit or GFJ may affect the intensity of the drug interaction.^{3, 9} Various GFJ products contain different levels of furanocoumarin constituents responsible for the inhibition of CYP3A; this could have resulted in divergent intensity of the interaction observed among studies. In previous reports, concentrations of bergamottin and DHB ranged from 1-37 μM and 0.2-52.5 μM respectively.^{9, 12, 32, 33} Many previous studies identified by our literature search did not describe the amounts of furanocoumarins present in the GFJ sample. The bergamottin and DHB concentrations stated in two identified studies by the systematic review were 23.5-33.1 μM and 2.7-6.7 μM respectively.^{25, 30} The HGJ used in this study contained low furanocoumarin amounts relative to values in juices studied in previously published reports (Table 1). None of the previous midazolam and GFJ interaction studies investigated use of HGJ containing low levels of furanocoumarin constituents. Importantly, to our knowledge, this is also the first study reporting the effects of hand-squeezed Hudson grapefruit juice on midazolam PK,

perhaps representing a more real-world grapefruit juice exposure, and the extent of CYP3A inhibition was smaller than in other studies.

Some limitations of this current study should be noted. Participants were healthy volunteers, and alteration of PK parameters may become more or less prominent in patients with certain disease states. One of the previous studies was conducted among patients with cirrhosis, and although direct comparisons cannot be made with healthy volunteers, the PK alteration appeared more pronounced. Extrapolation of current findings to patients with disease should be cautioned. The inhibition of CYP3A observed with RGJ in this study was less than reported in previous studies, which as noted may be related to the furanocoumarin contents of the juice used in this study.

In conclusion, this study found altered *in vivo* CYP3A activity by RGJ. The findings also suggest that CYP3A inhibition by GFJ that is low in furanocoumarin constituents may be minor. Importantly, GFJ does not always cause severe drug interaction with all medications that undergo CYP3A-mediated biotransformation. GFJ containing a negligible amount of furanocoumarin constituents may be a safer option if patients prefer to continue to drink grapefruit juice while taking drugs metabolized by CYP3A. To be cautious, however, grapefruit juice should be avoided with orally-dosed medications having low-bioavailability due to high presystemic extraction by CYP3A. It is noteworthy that the magnitude of grapefruit juice-drug interaction is not entirely consistent due to patient- and/or GFJ product-related variability as presented in this study and the currently available clinical evidence. If any medication is cautiously started at a lower-than-standard dose in patients who regularly consume GFJ, therapeutic effects should be monitored to adjust the dose appropriately. This is because the effect of GFJ may be less pronounced than expected due to certain patient- or GFJ product-related factors as observed in this study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Concentration-Time Curve for (A) midazolam and (B) 1'-hydroxymidazolam. The points represent mean values (N=12). The error bars represent standard deviations.

Figure 2.
Comparison of midazolam AUC. The points represent calculated AUC values for each study participant (N=12)

Figure 3.
Comparison of 1'-hydroxymidazolam $C_{\max}$. The points represent measured peak concentration values for each study participant (N=12)

Figure 4.
Comparison of 1'-hydroxymidazolam-to-midazolam metabolic ratios. The points represent calculated metabolic ratios for each study participant (N=12)

Table 1

Measurements of grapefruit juice (GFJ) constituents. Concentrations in μM (mean $\pm$ standard deviation).

GFJ	Bergamottin	6',7'-dihydroxybergamottin	Bergaptol	Isoimperatorin	Paradisic C	Spiroesters
Regular	43.4 $\pm$ 13.3	53.2 $\pm$ 3.8	4.0 $\pm$ 4.5	0.7 $\pm$ 0.4	11.1 $\pm$ 2.5	4.8 $\pm$ 2.3
Hybrid	1.8 $\pm$ 1.2	3.0 $\pm$ 1.3	0.5 $\pm$ 1.0	ND	0.1 $\pm$ 0.4	ND
Published Mean Value ¹⁸	23.3	69.3	1.3	48.1	9.9	5.5

ND=not detected.

Table 2

Summary of pharmacokinetic parameters. Values are shown as mean $\pm$ standard deviation.

	Water (Reference)	Regular-GFJ (Test)	GMR, % (90% CI)	Hybrid-GFJ (Test)	GMR, % (90% CI)
<i>Midazolam (MDZ)</i>					
AUC _{list} (ng·h/mL)	58.2 $\pm$ 23.3	67.7 $\pm$ 21.1	121 (105 – 139)	63.1 $\pm$ 20.8	111 (98 – 125)
AUC _{inf} (ng·h/mL)	64.8 $\pm$ 27.6	76.7 $\pm$ 27.5	122 (107 – 140)	70.2 $\pm$ 23.5	111 (98 – 125)
C _{max} (ng/mL)	25.1 $\pm$ 7.6	23.0 $\pm$ 7.1	92 (69 – 122)	22.3 $\pm$ 10.3	85 (68 – 107)
<i>1'-hydroxymidazolam (OHMDZ)</i>					
AUC _{list} (ng·h/mL)	28.2 $\pm$ 8.4	25.3 $\pm$ 6.8	91 (76 – 109)	26.2 $\pm$ 8.9	92 (81 – 104)
AUC _{inf} (ng·h/mL)	30.6 $\pm$ 8.3	28.0 $\pm$ 7.2	92 (79 – 108)	28.6 $\pm$ 9.9	92 (80 – 105)
C _{max} (ng/mL)	16.6 $\pm$ 10.4	9.7 $\pm$ 4.2	65 (42 – 100)	11.1 $\pm$ 6.5	68 (52 – 90)

GMR = geometric mean ratio; CI = confidence interval