Enzyme- and Transporter-Mediated Beverage–Drug Interactions: An Update on Fruit Juices and Green Tea

Guohua An, MD, PhD1, Jatinder Kaur Mukker, PhD2, Hartmut Derendorf, PhD2, and Reginald F. Frye, PharmD, PhD3

Abstract

Beverage–drug interactions have remained an active area of research and have been the subject of extensive investigations in the past 2 decades. The known mechanisms of clinically relevant beverage–drug interactions include modulation of the activity of cytochrome P450 (CYP) 3A and organic anion-transporting polypeptide (OATP). For CYP3A-mediated beverage–drug interaction, the in vivo CYP3A inhibitory effect is limited to grapefruit juice (GFJ), which increases the bioavailability of several orally administered drugs that undergo extensive first-pass metabolism via enteric CYP3A. In contrast, clinically significant OATP-mediated beverage–drug interactions have been observed with not only GFJ but also orange juice, apple juice, and, most recently, green tea. Fruit juices and green tea are all a mixture of a large number of constituents. The investigation of specific constituent(s) responsible for the enzyme and/or transporter inhibition remains an active area of research, and many new findings have been obtained on this subject in the past several years. This review highlights the multiple mechanisms through which beverages can alter drug disposition and provides an update on the new findings of beverage–drug interactions, with a focus on fruit juices and green tea.

Keywords

Beverage–drug interaction, Clinical Pharmacology (CPH), Fruit juices, Green tea, OATP, Pharmacokinetics and drug metabolism

As food/beverage and medications are often taken together, there is a high chance for food/beverage–drug interaction to occur. Food/beverages can influence drug disposition through multiple mechanisms. For example, a high-fat meal can slow gastric emptying and delay drug absorption. Food/beverages containing high amounts of metal ions (eg, milk and yogurt) can reduce the bioavailability of many antibacterial agents (eg, tetracycline, fluoroquinolone) through formation of insoluble chelates.1,2 Food/beverages rich in dietary fiber can increase the rate of drug absorption through facilitating gastric emptying and can decrease the extent of drug absorption by trapping coadministered drugs in the fiber matrix.3 In addition, food/beverages can also influence the disposition of coadministered drugs by modulating drug-metabolizing enzymes and/or transporters. The classic example of enzyme- and transporter-mediated food/beverage–drug interaction is with grapefruit juice (GFJ), a popular breakfast juice purchased by 21% of all households in the United States. Since the first clinical report in 1991,4 it has been well established that GFJ can interfere with presystemic metabolism of a number of therapeutic compounds (such as calcium channel blockers, statins, immunosuppressants, protease inhibitors),5–12 resulting in increased drug exposure and a corresponding increase in the incidence of adverse drug effects. Recently, GFJ was found to impair the bioactivation of clopidogrel, a prodrug dependent on CYP2C19- and CYP3A-mediated bioactivation to an active thiol metabolite; the interaction results in a decrease in the platelet-inhibitory effect of clopidogrel.13 Additional mechanistic studies have revealed that GFJ is an irreversible, mechanism-based inhibitor of intestinal CYP3A.14,15 The interaction of CYP3A substrate compounds with other fruit juices, including orange juice and apple juice, has also been investigated, and no interaction has been observed.5,16 Although many reports have documented the ability of GFJ to significantly increase the exposure of drugs that are CYP3A substrates, recent evidence has shown important interactions between GFJ and several drugs that undergo minimal metabolism.17–21 For example, when taken together with GFJ, the systemic exposure...
of fexofenadine\textsuperscript{19} and celiprolol\textsuperscript{18} were reduced substantially. This phenomenon cannot be explained by the known inhibitory effect of GFJ on CYP3A. These clinical observations stimulated further mechanistic studies that led to the discovery of a new mechanism of GFJ–drug interaction involving inhibition of organic anion-transporting polypeptides (OATPs), which are uptake transporters known to facilitate the uptake of substrate compounds through the gastrointestinal wall into the bloodstream. Interestingly, in addition to GFJ, several other fruit juices, including apple juice and orange juice, have also been found to influence the disposition of various drugs that are known to be substrates of OATPs.\textsuperscript{17,22–24} In addition to fruit juices, the interaction between drugs and green tea, another popular beverage consumed worldwide and often taken concomitantly with drugs, started to gain interest because of the recently observed modulatory effect of green tea on transporters, including OATPs.\textsuperscript{25–27} In 2010, the fruit juice retail market in the United States was valued at $16.2 billion. It has been estimated that the average American consumes around 30.3 L of fruit juice every year. In 2012, Americans consumed well over 3.60 billion gallons of tea, with 15% being green tea.\textsuperscript{28} Considering the extensive consumption of fruit juices and green tea, with 15% being green tea.\textsuperscript{28} Considering the extensive consumption of fruit juices and green tea, together with their modulatory effects on drug-metabolizing enzymes and transporters, there is potential risk for clinically important interactions between these beverages and coadministered drugs. GFJ–drug interaction has been extensively studied for more than 2 decades, and most beverage–drug interaction review articles have mainly focused on this subject.\textsuperscript{8,29–32} In the past few years, reports on the interactions of medications with other beverages, such as orange juice, apple juice, and green tea, have accumulated rapidly. In addition, fruit juices and green tea are all a mixture of a large number of constituents. The investigation of the specific constituent(s) responsible for the enzyme and/or transporter inhibition remains an active area of research, and many new findings have been obtained on this subject in the past several years (Table 1).\textsuperscript{33–36} This review covers the multiple mechanisms that have been evaluated for beverage–drug interaction, including CYP3A, P-glycoprotein, and OATPs and provides an update on new findings of beverage–drug interactions, with a focus on fruit juices and green tea.

### CYP3A-Mediated Beverage–Drug Interaction

#### Grapefruit Juice

GFJ combines the sweet and tangy flavors of the orange and the pomelo (or shaddock) and provides high amounts of vitamin C and potassium. GFJ carries the American Heart Association’s healthy “heart-check” food mark and is consumed widely as a preventive measure against

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<th>CYP3A Inhibition</th>
<th>P-gp Inhibition</th>
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\*Coincubation effect. \#Preincubation effect. X, no effect; +, weak effect; ++, moderate effect; +++, strong effect; N/A, information unavailable; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.
The data from this study were published in the literature with the same felodipine dose. This discrepancy led to a subsequent clinical study conducted in 6 men to evaluate the role of GFJ on felodipine disposition. The results showed that GFJ significantly increased the oral bioavailability of felodipine and tripled the felodipine area under the concentration–time curve (AUC) compared with water. The data from this study were published in the *Lancet* in 1991 and represent the first reported clinical food/beverage–drug interaction. Since then, the GFJ–drug interaction has been investigated extensively, and numerous clinical studies have documented clinically relevant interactions between GFJ and coadministered drugs. For example, GFJ enhanced the AUC of saquinavir by 220% compared with water. GFJ also significantly increased the exposure of simvastatin. In 2 studies conducted in healthy volunteers, GFJ augmented the cyclosporine AUC to 162% and 143% of that with water. GFJ also significantly altered terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. Similar interactions have been documented for GFJ and nisoldipine, nitrendipine, lovastatin, atorvastatin, midazolam, cisapride, and several others. Several similarities were noticed in these reports. First, in most cases the drugs on which GFJ had a significant effect were typically CYP3A substrates with a high first-pass metabolism. Second, for those drugs on which GFJ had a significant effect, GFJ had minimal impact on their half-life, indicating that the increased systemic exposure of coadministered drugs was mainly caused by inhibition on their first-pass metabolism, resulting in the increase in drug bioavailability. Third, clinically significant GFJ interactions were only observed when the drugs were administered orally. No interaction was observed when the same drug was given by intravenous administration. For example, the effect of GFJ was evaluated on the disposition of felodipine, cyclosporine, and midazolam after both intravenous and oral administration of these drugs. Significant interactions were observed when these drugs were taken orally; GFJ had no effect on the pharmacokinetics of felodipine, cyclosporine, and midazolam when they were given intravenously. These observations indicated that GFJ might interact with coadministered drugs through inhibition of intestinal CYP3A, resulting in the reduction of gut wall metabolism and an increase in drug bioavailability.

The direct evidence of intestinal CYP3A inhibition by GFJ was provided by several in vitro and in vivo studies. For example, Watkins and coworkers evaluated the effect of repeated GFJ ingestion on CYP3A expression in healthy male volunteers. In their study, before and after receiving GFJ, small bowel and colon mucosal biopsies were obtained by upper intestinal endoscopy. The protein concentrations of CYP3A and several other CYP isofoms, including CYP3A5, CYP1A1, and CYP2D6, were measured. In addition, liver CYP3A activity was measured with the erythromycin breath test in each subject. Their results showed that GFJ decreased enterocyte CYP3A protein concentrations in every subject. After receiving GFJ for 6 days, the mean enterocyte CYP3A protein concentration decreased 62%. In contrast, GFJ did not alter liver CYP3A activity, which is in line with the clinical observations that GFJ has no impact on the systemic clearance of CYP3A substrates. In addition, GFJ did not alter colon levels of CYP3A5, or small bowel concentrations of CYP1A1 and CYP2D6. In addition to measuring the expression of these proteins after repeated doses of GFJ, the authors also evaluated the acute effect of GFJ, and the preliminary data demonstrated that intestinal CYP3A protein concentration can be greatly reduced (decreased by 47%) within 4 hours of consumption of a single glass of GFJ. The authors also examined the effect of GFJ on CYP3A mRNA expression and found that the mean enterocyte CYP3A mRNA concentration was unchanged after 6 days of GFJ intake. Based on the observation of reduced small bowel CYP3A protein concentration with unchanged CYP3A mRNA expression, the possible explanation of the GFJ effect was decreasing CYP3A protein content through a posttranscriptional mechanism, possibly involving accelerated CYP3A protein degradation via irreversible mechanism-based enzyme inhibition.

As GFJ can markedly decrease the intestinal CYP3A protein content, many researchers anticipated that GFJ may have a prolonged inhibitory effect on CYP3A because the return of CYP3A activity is dependent on de novo enzyme synthesis. With this hypothesis, several clinical studies were conducted to evaluate the duration of activity of GFJ. In one study, the relationship between time of intake of GFJ and its effect on felodipine pharmacokinetics was evaluated in 9 healthy subjects. Consumption of a single glass of GFJ 0, 1, 4, 10, or 24 hours before felodipine administration showed that the magnitude of increase in felodipine exposure was maximal with consumption of GFJ 0, 1, or 4 hours before its administration. The magnitude of GFJ effect declined gradually with increasing time interval between GFJ and felodipine administration. However significantly higher felodipine C_max was still observed, even when GFJ was consumed 24 hours before felodipine. In another study, the duration of GFJ effect was evaluated on...
nisoldipine pharmacokinetics with similar study design. In that study, various time intervals of up to 4 days were evaluated between GFJ and nisoldipine administration. The results showed that the effect of GFJ decreased in a time-dependent manner and lasted for at least 3 days after intake. Therefore, for those drugs with a narrow therapeutic window and known to interact with GFJ and for elderly people, who are less tolerant of fluctuation in drug exposure, GFJ might need to be avoided for 72 hours before taking those medications.

In addition to a prolonged effect, another feature of GFJ–drug interaction is that the magnitude of the pharmacokinetic interaction is highly variable among individuals. For example, Watkins and coworkers observed that the magnitude of the increase in felodipine exposure produced by GFJ varied substantially in 10 healthy volunteers, with felodipine C_max ranging from 64% to 597% and AUC from 4% to 368%. The same research group also found substantial interindividual differences in the concentrations and activities of CYP3A in small bowel enterocytes. A further relationship between CYP3A content and felodipine exposure was examined, and the result revealed that subjects with the highest content of intestinal CYP3A before GFJ administration tended to have the largest fall in CYP3A content and the greatest increase in felodipine plasma concentration with GFJ coadministration. Therefore, the large variability of GFJ effect observed among individuals within each clinical study is dependent on inherent differences in baseline intestinal CYP3A protein content. In addition to the large variability of GFJ effect among individuals within the same study, variable results were also obtained when the mean GFJ effect was compared across different clinical studies. Variability in the concentrations of GFJ constituents across different studies may represent an important factor, because those constituents are known to be dependent on a number of factors, including the type and origin of the grapefruits used, the manufacturing process, and storage conditions. Another possible factor contributing to the variable results across different clinical studies could be the variation in study design, such as double-strength versus single-strength GFJ administration. It appears that double-strength GFJ can produce a much greater effect than regular-strength GFJ. For example, Rogers et al. reported that the AUC of lovastatin increased 1.94-fold after consumption of regular-strength GFJ. In contrast, Kantola et al. found that double-strength GFJ increased the AUC of lovastatin about 15.3-fold. Similarly, the AUC of simvastatin increased in a much more pronounced manner in the presence of double-strength GFJ (16-fold) than in the presence of regular-strength GFJ (3.6-fold). In addition to the different strengths of GFJ used, another variation in study design among different clinical studies is a single glass of GFJ (acute exposure) versus repeated GFJ ingestion (extended exposure). The available reports are inconsistent regarding whether repeated GFJ ingestion can cause a cumulative inhibitory effect. For example, Culm-Merdek et al. reported that acute and extended exposure to GFJ increased the AUC of triazolam by 51% and 60%, respectively, and the difference was not statistically significant. Similarly, Lundahl et al. compared the effect of GFJ on felodipine pharmacokinetics after 1 glass of GFJ or repeated GFJ ingestion for 14 days, and they found that the interaction effect of GFJ was maximal after the first glass. On the other hand, Lown et al. found that the AUC of felodipine increased about 2.2-fold after the first 8-oz glass of GFJ, but 3.1-fold after 5 days of thrice-daily administration of GFJ. Further investigations are needed to have a better understanding of whether GFJ has a cumulative CYP3A inhibitory effect.

In addition to the variations described above, there is a difference between the theoretically predicted risk and clinically relevant and well-documented GFJ–drug interactions. The literature provides generalized theoretically predicted information on different classes of drug–GFJ interactions, which may mislead readers to avoid coadministration of GFJ and drugs whose interactions are negligible and/or clinically unimportant such as quinidine, oxycodone, nilotinib, and sunitinib.

Numerous studies have been conducted to evaluate the CYP3A-mediated GFJ–drug interactions. Detailed lists of evaluated drugs, including both affected and unaffected ones, have been summarized in a number of excellent review articles. In addition, the research center at the University of Florida, Food Drug Interaction Research & Education (http://www.DrugInteractionCenter.org), provides an up-to-date list of drugs that have been evaluated, including the interaction magnitude and sources of relevant scientific literature to support clinically documented GFJ interactions.

It should be noted that in addition to the magnitude of the increase in the exposure of coadministered drugs, additional factors such as the severity of toxicities of the affected drug need to be considered as well. For those drugs with narrow therapeutic windows and that undergo first-pass metabolism through intestinal CYP3A, GFJ should be avoided even if the magnitude of interaction on the pharmacokinetic side is minimal or mild. Several case reports documented drug toxicity that was possibly caused by a large amount and long-term consumption of GFJ or grapefruit. For example, Peynaud et al. reported nephrotoxicity of tacrolimus after consumption of a large amount of marmalade that contained grapefruit (1.5 kg eaten during the preceding 1 week). Goldbart et al. reported myelotoxicity of colchicine after consumption of juice 1 L/day for the preceding 2 months. The limitation of the case reports is that the patients took multiple drugs together, and there is no information available regarding
the concentration of the drug with toxicity. As a result, it is difficult to conclude that the drug toxicity was truly caused by the pharmacokinetic interaction between that drug and GFJ. For example, Karch reported rhabdomyolysis of atorvastatin, and GFJ was anticipated to be the culprit because the patient drank 2–3 glasses of GFJ (extracted from fresh fruit) daily for 2 months. However, the concentration of atorvastatin was not measured in this case report. On the other hand, results from a controlled study conducted in 130 patients showed that there is no clinically significant interaction following concomitant administration of atorvastatin and a moderate amount of GFJ (300 mL daily) for 90 days.

Other Fruit Juices
After the CYP3A-mediated GFJ–drug interaction was recognized, the effect of other fruit juices on the disposition of CYP3A substrates was evaluated in several clinical studies. The interaction between orange juice and felodipine was evaluated in healthy volunteers, and the results revealed that orange juice does not affect the pharmacokinetics of felodipine. Similarly, results from another clinical study showed that apple juice did not significantly affect the pharmacokinetics of midazolam, indicating no effect on CYP3A in humans. On the other hand, juice from pomelo grapefruit (Citrus paradisi Macf., G) has been reported to increase the oral bioavailability of felodipine. In addition, Seville (sour) orange juice was found to increase the felodipine AUC to the same extent observed with GFJ. A significant increase in dextromethorphan exposure with Seville (sour) orange juice has also been reported. In vitro mechanistic studies indicated that Seville (sour) orange juice decreased intestinal CYP3A protein concentration and therefore might inhibit CYP3A by the same mechanism as GFJ. Regarding CYP3A-mediated fruit juice–drug interaction, although both lime juice and blueberry juice demonstrated an inhibitory effect on CYP3A activity in vitro, both of them showed negative results in vivo when they were evaluated with felodipine and buspirone, respectively, in 2 independent clinical studies. However, it should be noted that the lime juice evaluated in vivo was a diluted one (one-quarter strength), and although mean felodipine exposure was not changed in the presence of dilute lime juice, the magnitude of felodipine increase was correlated with that observed with grapefruit juice among individuals ($r^2 = 0.95$). In addition, Kim et al evaluated the effects of various fruit juices on the activity of midazolam 1'-hydroxylase, a marker of CYP3A, in pooled human liver microsomes. Their results demonstrated that black mulberry juice showed the most potent inhibition of CYP3A (except for grapefruit juice). The inhibitory potential on human CYP3A was in order GFJ > black mulberry juice > wild grape juice > pomegranate juice > black raspberry juice. Whether their CYP3A inhibitory effect is clinically relevant warrants further in vivo investigation.

Green Tea
Made from the leaves of Camellia sinensis, green tea is the most widely consumed beverage in the world. It can be found in almost 80% of all US households. There are several types of tea based on the difference in various degrees of processing and the level of oxidization. Green tea is not oxidized after leaf harvesting, so it most closely resembles the chemical composition of the fresh tea leaf. Green tea has become the raw material for extracts used in various beverages, health foods, and dietary supplements. The effect of green tea on CYP3A activity has been investigated in both in vitro and in vivo studies. Misaka et al evaluated the effect of green tea extract (GTE) on the activity of CYP3A in vitro using pooled human intestinal and liver microsomes. A noncompetitive inhibitory effect of GTE on CYP3A was observed, with IC$_{50}$ of 18.4 µg/mL in human intestinal microsomes and 13.8 µg/mL in human liver microsomes. In another study, GTE from various manufacturers was compared in terms of the effect on CYP3A. An in vitro study was conducted in human liver microsomes using quinine as the CYP3A probe drug. The results showed that the GTE inhibited CYP3A, with the inhibition ranging from 5.6% with Nature’s Resource to 89.9% with Natrol GTE. The effect on CYP3A varied among different brands of GTE, possibly because of variations in the content of the herbal product’s active ingredients. In addition to in vitro studies, CYP3A-mediated green tea–drug interaction has also been evaluated in animal studies. For example, following a single oral dose of GTE (400 mg/kg) in rats, simvastatin AUC increased 3.4-fold compared with that in the simvastatin alone group. It is worth pointing out that the GTE–simvastatin interaction observed in rats may not be easily translated into human because, in general, rats are not considered as appropriate animal models for human CYP3A4 predictions. It has been reported that the inhibition and induction of the major rat CYP3A isoform in liver (CYP3A1) do not resemble human CYP3A4. So far, no clinically significant interaction between green tea and CYP3A substrates has been observed in clinical studies. For example, 4 weeks of green tea catechin intervention only resulted in a 20% increase in buspirone exposure, suggesting a small reduction in CYP3A activity. Overall the clinical data on CYP3A-mediated green tea–drug interaction is quite limited. Whether the inhibitory effect of green tea on CYP3A is clinically relevant warrants further investigation.

Candidate Constituents for CYP3A Inhibition
In addition to vitamin C and potassium, the major constituents in GFJ are the flavonoids, with naringin in...
most abundant. Naringin is responsible for the bitter taste of GFJ and has been found to be present in GFJ at concentrations up to 1200 mg/mL. In addition to naringin, other flavonoids, such as quercetin and kaempferol, are also present in GFJ in trace amounts. Although naringin is prevalent in GFJ, it is absent from orange juice. As orange juice does not affect CYP3A, people initially suspected that naringin might be the ingredient in GFJ causing CYP3A inhibition. However, commercially available pure naringin administered in the same amount as found in GFJ produced little change in the pharmacokinetics of nisoldipine or felodipine, indicating that naringin is not the main contributor to CYP3A-mediated GFJ–drug interactions. Quercetin, another flavonoid present in GFJ and many other juices, was found to have an inhibitory effect on CYP3A in vitro. However, a clinical study conducted in 8 healthy volunteers showed that after an oral dose of 400 mg quercetin, nifedipine exposure was not changed, indicating that quercetin may not be responsible for interactions as well. As flavonoids present in GFJ failed to explain the in vivo CYP3A-mediated GFJ–drug interaction, further investigation was conducted to evaluate other constituents of GFJ. In addition to flavonoids, GFJ also contains furanocoumarins, including 6',7'-dihydroxybergamottin and bergamottin. The effect of 6',7'-dihydroxybergamottin on CYP3A has been evaluated in several in vitro studies. Edwards et al. found that 6',7'-dihydroxybergamottin has a potent inhibitory effect on CYP3A, and the addition of 6',7'-dihydroxybergamottin to orange juice decreased CYP3A activity to values comparable to those observed with GFJ. Studies conducted by Watkins and coworkers using recombinant CYP3A revealed that 6',7'-dihydroxybergamottin is a mechanism-based inhibitor and caused time- and concentration-dependent inactivation of CYP3A. This finding supports the idea that loss of intestinal CYP3A produced by GFJ in vivo may result from accelerated degradation of the enzyme. Based on these in vitro investigations, 6',7'-dihydroxybergamottin is considered an active ingredient in GFJ on CYP3A inhibition. In addition to 6',7'-dihydroxybergamottin, bergamottin, another major furanocoumarin present in GFJ, was also evaluated clinically, and the data indicated that bergamottin was also active. In addition to 6',7'-dihydroxybergamottin and bergamottin, dimers of the furanocoumarins, also known as paradsins, have also demonstrated a potent CYP3A inhibitory effect in several in vitro studies. To confirm whether furanocoumarins are the main contributors of the in vivo CYP3A-mediated GFJ–drug interactions, several studies compared the effect of modified GFJ (furanocoumarins were removed) with the original GFJ. The results showed that furanocoumarin-free GFJ failed to alter the disposition of felodipine and cyclosporine, 2 drugs known to interact with unmodified GFJ. With the role of furanocoumarins in CYP3A-mediated GFJ–drug interactions confirmed, efforts have been taken recently to remove furanocoumarins from GFJ based on the chemical, physical, and genetic approaches. For example, it has been reported that furanocoumarins can be removed by chemical extraction and reconstitution, degraded by heat, or inactivated by ultraviolet radiation (Figure 1). In addition, Gmitter and coworkers used genetic modification to develop GFJ cultivars with low or free of furanocoumarins (Figure 2).

Other than GFJ, only a few other fruit juices, such as Seville (sour) orange juice and pomelo grapefruit juice have been reported to have significant in vivo interaction with CYP3A substrates. Similar to GFJ, Seville (sour) orange juice and pomelo grapefruit juice also contain furanocoumarins. Therefore, furanocoumarins may be the active ingredients in Seville (sour) orange juice and pomelo grapefruit juice responsible for the observed in vivo interactions.

### P-gp-Mediated Beverage–Drug Interaction

P-glycoprotein (P-gp) is one of the most important members of the ATP-binding cassette transporter superfamily, and it has been extensively studied since it was discovered by Ling and coworkers in 1976. P-gp was initially discovered in cancer cells and represents an important mechanism of multidrug resistance. Further investigation on localization of P-gp revealed that P-gp is not confined to tumor cells; it is also expressed in a number of normal human tissues, including intestine, liver, kidneys, and brain and has been found to play a pivotal role in drug absorption, distribution, and elimination. Variably clinically important compounds, including anticancer drugs, HIV protease inhibitors, immunosuppressive agents, H2-receptor antagonists, and antibiotics, have been reported to be substrates of P-gp. Similar to CYP3A, P-gp is also highly expressed in the enterocytes of the intestine and hepatocytes of the liver, where it actively pumps various xenobiotics back into the gastrointestinal tract, thereby preventing absorption into systemic circulation. CYP3A and P-gp have been found to have substantial overlap in substrates and modulators and are believed to play a concerted role in drug disposition. The investigation of the interplay between intestinal CYP3A and P-gp remains an active area of research.

### Grapefruit Juice

Because many CYP3A modulators are also found to have a modulatory effect on P-gp, the potent inhibitory effect of GFJ on intestinal CYP3A led many research groups to evaluate the effect of GFJ on P-gp. Takanaga et al. investigated the effect of GFJ extracts on the transport of
vinblastine, a well-known P-gp substrate, across Caco-2 cells. They observed enhanced uptake and apical-to-basolateral transport of vinblastine in the presence of GFJ extracts, indicating an inhibitory role of GFJ on P-gp.98 Similarly, Butterweck and coworkers reported that the basolateral-to-apical directional transport of talinolol, a nonmetabolized P-gp substrate, was selectively inhibited by GFJ and its components.100 Benet’s group, on the other hand, reported contradictory findings when they evaluated the effect of GFJ with a range of concentrations (0.05%, 0.5%, and 5%) on the transport of vinblastine across MDCK/MDR cell monolayers.99 They observed a significant increase in the basolateral-to-apical transport of vinblastine in the presence of GFJ, indicating a possible stimulation role of GFJ on P-gp.99 In addition, they found that GFJ activated the efflux of vinblastine in a concentration-dependent manner. Interestingly, Mitsunaga et al. found mixed results in a study evaluating the effect of citrus bioflavonoids on vincristine transport across MBEC4 (mouse brain capillary endothelial) cells.103 In the concentration range of 10–50 μM, quercetin and kaempferol (both present in GFJ) showed

Figure 1. (A) Nifedipine plasma concentration–time curves after intraduodenal administration of nifedipine 30 minutes after preadministered saline, GFJ, and 6-hour ultraviolet-irradiated GFJ. (B) Concentration of bergamottin and 6,7-dihydroxybergamottin (6,7-DHB) in GFJ after 6-hour ultraviolet irradiation. A and B adapted from Uesawa Y, Mohri K. Biol Pharm Bull, 2006;29:1286–1289.

Figure 2. Concentration of 6,7-DHB, bergamottin, and paradisin C in the diploid hybrids. The 3 furanocoumarins (FCs) in half of them were undetectable or extremely low. This figure is adapted from Chen C et al. J. Amer Soc Hort Sci. 2011;136:358–363.
concentration-dependent biphasic modulation of vinristine uptake by activating P-gp at low concentrations (10 μM) and inhibiting P-gp at high concentrations (50 μM). A potential explanation for these disparate results is that different research groups used different experimental conditions. The concentrations of GFJ tested in Benet’s group were low, and the stimulatory effect of GFJ on P-gp observed at those low GFJ concentrations appears to be in line with the phenomenon of biphasic modulation of flavonoids on P-gp reported by Mitsunaga et al. In addition to P-gp activity, the effect of GFJ on P-gp expression was also investigated by several research groups, and conflicting results were obtained, ranging from decreasing P-gp level to no effect, to increasing P-gp level. For example, Watkins and coworkers found that consumption of GFJ for 6 days did not affect intestinal P-gp protein content in 10 healthy volunteers. Romiti et al. evaluated the effect of GFJ on the expression of P-gp in human tubular cell line HK-2, and they observed a dose-dependent decrease in both P-gp protein level and mRNA level in the presence of GFJ. However, Panchagnula et al. found that chronic administration of GFJ led to increased levels of P-gp expression in rat everted ileum sac. It should be noted that among above 3 studies, only Watkins’s group evaluated the in vivo effect of GFJ on intestinal P-gp expression in humans, and the remaining 2 studies were performed either in vitro in a kidney cell line or in vivo in rat. In addition to in vitro investigations, several preclinical and clinical studies were conducted to evaluate the effect of GFJ on the disposition of drugs that are P-gp substrates with minimal CYP3A-mediated metabolism. One clinical study investigated the interaction between GFJ and digoxin, a well-known P-gp substrate undergoing little metabolism. The results demonstrated that GFJ did not alter the bioavailability of digoxin. However, as digoxin has high oral bioavailability (70%–80%), whether it is an appropriate P-gp probe drug to be evaluated in vivo is questionable. Another study evaluated the effect of GFJ on the disposition of talinolol in rats, another nonmetabolized P-gp substrate, and found that in the presence of GFJ, the exposure of talinolol was doubled. The clinical significance of P-gp-mediated GFJ–drug interactions remains to be elucidated.

Other Fruit Juices
In addition to GFJ, the effect of several other fruit juices on P-gp has also been investigated. For example, although orange juice does not affect CYP3A activity or expression, a 50% ethyl acetate extract of orange juice was found to significantly decrease the efflux ratio of vinblastine and saquinavir in Caco-2 cells, indicating an inhibitory effect of orange juice extract on P-gp. Seville (sour) orange juice has been suggested to selectively inhibit intestinal CYP3A without affecting P-gp based on the observations that it increases the exposure of felodipine but not that of cyclosporine. Xu et al. evaluated the effects of several citrus fruit juices on the transport of digoxin in Caco-2 cell monolayers and showed that the apical-to-basolateral digoxin efflux was enhanced 50% by fruit juice in the order of lemon juice > lime juice > pomelo juice > GFJ. Most investigations of P-gp-mediated interaction between drugs and fruit juices (other than GFJ) have been mainly conducted in vitro. Thus, the in vivo effect of fruit juices on P-gp remains unclear and warrants further evaluation.

Green Tea
The modulatory effects of green tea catechins on P-gp have been observed in many independent studies. For example, Qian et al. evaluated the effect of epigallocatechin gallate (EGCG), the most abundant catechin in green tea, on P-gp activity. A 3-dimensional model of the carboxyl-terminal nucleotide-binding domain (NBD2) form of P-gp was built by homology modeling. The structural model of the complex indicated that EGCG was tightly bound to the ATP-binding site of NBD2. In addition, results of the in vitro uptake study showed that EGCG inhibited P-gp and increased the intracellular accumulation of doxorubicin in drug-resistant KB-A1 cells. The authors also conducted an in vivo study using nude mice bearing doxorubicin-resistant P388 leukemia and found that EGCG could enhance the efficacy of doxorubicin and increased the doxorubicin concentration in the resistant tumors. Jodoin et al. reported that green tea catechins, at the concentration of 30 μg/mL, inhibited the photo-labeling of P-gp by 75% and increased by 3-fold the accumulation of rhodamine-123 in the multidrug-resistant CH9C5 cell line. Moreover, the modulation of P-gp transport by green tea catechins was found to be a reversible process. Among the catechins tested, EGCG, epicatechin gallate (ECG), and catechin gallate were all found to have inhibitory effects on P-gp. Similarly, another study evaluated the effects of individual green tea catechins and found that several catechins inhibit P-gp, with a potency rank order of EGCG > ECG > EGC. In contrast, epicatechin (EC) did not have any effect on P-gp. Although the inhibitory effect of green tea catechins on P-gp has been demonstrated in several in vitro and in vivo animal studies, the clinical relevance of the interaction between green tea catechins and P-gp substrates is unknown and remains to be elucidated.

Candidate Constituents for P-gp Inhibition
In addition to fruit juices or fruit juice extract, the individual constituents have also been studied to identify the candidate active ingredients for P-gp modulation. Naringin is a major constituent in GFJ that has been reported to reduce the apical efflux of vinblastine at the
concentration present in GFJ and increase steady-state uptake from the apical side to 124%. However, the effect of naringin is modest and may not account for the effect observed with GFJ. Bergamottin and 6',7'-dihydroxybergamottin, the major furanocoumarins present in GFJ, have also been evaluated, and both ingredients were found to decrease the efflux ratio of vinblastine and saquinavir in bidirectional transport studies conducted in Caco-2 cell monolayers, indicating an inhibitory effect on P-gp. In addition, quercetin and kaempferol, both present in GFJ, were found to have a biphasic effect on P-gp in vitro, and both function as stimulators at low concentrations and inhibitors at high concentrations. On the other hand, Kim et al. evaluated the in vivo effect of quercetin on the pharmacokinetics of fexofenadine in healthy volunteers, and they found that short-term use of quercetin elevated the plasma concentrations of fexofenadine (60% increase in fexofenadine AUC), probably by the inhibition of P-gp-mediated efflux in humans. In addition to constituents of GFJ, components from orange juice have also been evaluated regarding their modulatory effect on P-gp. The polymethoxylated flavones in orange juice, including 3,3',4',5,6,7,8,-heptamethoxyflavone (HMF), 4',5,6,7,8,-pentamethoxyflavone (tangeretin), and 3',4',5,6,7,8,-hexamethoxyflavone (nobiletin) were found to increase the steady-state uptake of vinblastine in Caco-2 cells in a concentration-dependent manner. The order of potency of these compounds on P-gp inhibition at a concentration of 50 μM was tangeretin > HMF > nobiletin. Butterweck and coworkers also evaluated the effect of polymethoxylated flavones from citrus on P-gp using talinolol as a probe substrate. They found that polymethoxylated flavones significantly decreased talinolol transport from the basolateral to the apical side, with tangeretin being the most potent, followed by nobiletin, HMF, and sinensetin. In contrast, none of these polymethoxylated flavones inhibited CYP3A. In addition, it has been reported that the P-gp inhibitory effect of GFJ components, including bergamottin and 6',7',9'-dihydroxybergamottin, were considerably weaker than those polymethoxylated flavones in orange juice. Regarding green tea, the information related to its candidate active ingredients on Pgp is discussed in the Green Tea section above.

**OATP-Mediated Beverage–Drug Interaction**

OATPs belong to the family of membrane solute carrier (SLC) transporters and have been found to facilitate the sodium-independent influx of both endogenous and exogenous compounds. Opposite to the function of P-gp and other efflux transporters, which pump the drugs out of cells, OATPs facilitate the translocation of drugs into cells and therefore are considered uptake transporters. OATPs are encoded by solute carrier organic anion-transporting (SLC01) genes and up to now 11 human OATP transporters have been identified, among which OATP1A2, OATP1B1, OATP1B3, and OATP2B1 have been well characterized. These 4 OATPs have considerable overlap in their substrate spectrum, and they transport various compounds from different classes, including angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, antihistamines, statins, antibiotics, and beta-adrenergic blocking agents. Although both lipid-soluble drugs and water-soluble drugs can be transported by OATPs, intestinal OATPs usually play a bigger role in the absorption of hydrophilic drugs than do lipophilic drugs, as the latter can also undergo other pathways (such as passive diffusion) for absorption and consequently will not be influenced as dramatically as the hydrophilic drugs when the intestinal OATPs are inhibited. The expression of OATP1B1 and OATP1B3 is confined to the liver, and these 2 transporters have been reported to play an important role in hepatic uptake of xenobiotics across the sinusoidal membrane from blood. In contrast to the tissue-specific expression of OATP1B1 and OATP1B3, OATP2B1 is localized in several tissues, including liver, small intestine, spleen, and placenta. OATP2B1 is expressed in significant amounts on the apical membrane of small intestinal epithelial cells, thereby facilitating the transfer of its substrates from the intestine to the bloodstream. Similar to OATP2B1, OATP1A2 is also expressed in several tissues, including brain, testis, and prostate. The function of OATP1A2 in the intestine has been debated in the literature because of the controversial reports on its expression in the intestine. Some studies reported no OATP1A2 detection, whereas other groups identified relatively low SLCO1A2 mRNA expression in the intestine.

**Grapefruit Juice**

The modulatory effect of GFJ on OATPs was discovered unexpectedly from several clinical studies in which the original aim was to evaluate the role of GFJ in the disposition of drugs that are substrates of P-gp. As GFJ has demonstrated the inhibitory effect on P-gp in many in vitro studies, several research groups conducted further clinical studies to test the hypothesis that GFJ would enhance the oral bioavailability of nonmetabolized P-gp substrates in vivo. However, 2 independent clinical studies, one conducted by Parker et al. and another by Becquemont et al., showed that in the presence of GFJ, the change in the pharmacokinetics of digoxin, a P-gp substrate undergoing minimal metabolism, was rather modest. One potential explanation for this negative result is the intrinsically high oral bioavailability of digoxin, which makes the interpretation difficult. This stimulated a search for more suitable P-gp probe drugs to
have better evaluation of P-gp-mediated GFJ–drug interactions. Fexofenadine, an antihistamine drug, and celiprolol, a selective β1-adrenergic receptor-blocking agent, were considered suitable P-gp probe drugs. Both drugs are substrates of P-gp, and their metabolism is negligible in humans, meaning that the GFJ effect on CYP3A would not confound the results. In addition, P-gp inhibitors (eg, itraconazole, ritonavir) have been reported to increase the exposures of fexofenadine and celiprolol, suggesting that P-gp plays an important role in their absorption.18,130–132 More importantly, both compounds have relatively low bioavailability (~30%), which means that the interaction would not be masked if GFJ truly affects P-gp. However, when the effect of GFJ on the disposition of fexofenadine and celiprolol was evaluated separately in healthy volunteers by 2 independent research groups (one by Lilja et al. and another by Dresser et al.),18,19 similar results were obtained: opposite to what they expected, that the exposure of these 2 drugs would increase with GFJ ingestion, the exposures of both fexofenadine and celiprolol were significantly decreased. Dresser et al. found that the AUC and \(C_{\text{max}}\) of fexofenadine in the GFJ coingestion group were reduced to about 33% and 38%, respectively, of the values obtained in the control group.19 Similarly, Lilja et al. reported that during the GFJ phase, the AUC and \(C_{\text{max}}\) of celiprolol were substantially reduced to about 13% and 5%, respectively, of the respective placebo phase values.18 Consistent with these observations, GFJ was later found by Schwrz et al. to also be able to decrease the plasma concentrations of talinolol.133 As these phenomena cannot be explained by the known inhibitory effect of GFJ on P-gp (or CYP3A), the researchers conducting these studies anticipated that these interactions might be mediated by a novel and potentially clinically important mechanism. Further investigations revealed that these compounds are substrates of OATPs, an uptake transporter family that can enhance the bioavailability of substrate compounds through facilitating their uptake from the intestine. Among the OATP members, initially OATP1A2 was proposed by Kim and coworkers as the key intestinal uptake transporter for the GFJ–fexofenadine interaction.19,134 They screened OATP uptake transporters and found that only OATP1A2 can effectively transport fexofenadine.134 The same research group also examined the expression of a panel of uptake transporters using human duodenal biopsy samples and detected the mRNA expression of OATP1A2. Recently, Nozawa et al. reported that fexofenadine, along with several other drugs, is transported by OATP2B1 at both neutral and acidic pH.135 The role of OATP2B1 in fexofenadine absorption was further confirmed by an in vivo clinical study. It has been reported that there is a single nucleotide polymorphism in human OATP2B1, which causes a change in function (c.1457C>T, S486F).136 Imanaga et al. investigated the effect of the SLCO2B1 c.1457C>T polymorphism on fexofenadine pharmacokinetics and found that when fexofenadine was administered with water, subjects with the c[1457C>T] allele showed a significant decrease in fexofenadine AUC with no change in half-life, indicating an important role of OATP2B1 in fexofenadine absorption.16 Considering the high expression of OATP2B1 in the intestine and its important role in the uptake of fexofenadine and other OATP substrates, OATP2B1 may play a bigger role than OATP1A2 in OATP-mediated GFJ–drug interactions. The contribution of OATP1A2 to in vivo OATP-mediated GFJ–drug interactions warrants further investigation.

Interactions between GFJ and CYP3A substrates are known to occur via irreversible mechanism-based inhibition, leading to a prolonged effect of GFJ on CYP3A inhibition.14,15 Regarding duration of activity of GFJ on OATP, Kim and colleagues reported that consumption of GFJ concomitantly or 2 hours before fexofenadine administration was associated with reduced oral fexofenadine plasma concentrations, whereas fexofenadine exposure was not affected when GFJ was taken 4 hours before drug administration.134 Recently, Tanaka et al. compared the inhibitory duration of GFJ on celiprolol (an OATP substrate) and midazolam (a CYP3A substrate) in healthy volunteers, and their results also indicated that GFJ inhibition on OATP appears to dissipate over a shorter period than has been observed with CYP3A inhibition.137 In addition to the inhibitory duration of GFJ on OATP, the effect of GFJ volume on OATP inhibition was also evaluated.138 Bailey and coworkers conducted a randomized 4-way crossover study in which GFJ or water at normal (300-mL) or high (1200-mL) volume was ingested concomitantly with 120 mg fexofenadine in 12 healthy volunteers.138 Their results revealed that the 300- and 1200-mL volumes of GFJ diminished the mean exposure of fexofenadine to 58% and 36%, respectively, of those observed with equivalent volumes of water.138 As a 4-fold higher GFJ volume only reduced fexofenadine AUC moderately more, a commonly consumed volume (300 mL) of GFJ appeared to be near the upper portion of the volume–response curve and already produced a clinically significant magnitude of OATP inhibition. To date, many clinical studies have been carried out to evaluate OATP-mediated GFJ–drug interactions. Lists of evaluated drugs, including both affected and unaffected ones, have been summarized in a number of excellent review articles.20,21

**Other Fruit Juices**

In contrast to CYP3A-mediated fruit juice–drug interactions, in which orange juice and apple juice do not show in vivo CYP3A inhibitory effects, clinically significant OATP-mediated fruit juice–drug interactions were...
observed not only with GFJ, but also with orange juice and apple juice (Figure 3).16,19 Dresser et al. examined the effect of GFJ, orange juice, and apple juice on the disposition of fexofenadine in healthy volunteers, and their results revealed that all these 3 fruit juices markedly decreased fexofenadine exposure, with the reduction in plasma concentration of fexofenadine being the largest in the case of coadministration with apple juice, followed by orange juice and GFJ.19 This indicated that apple juice has a more potent in vivo inhibitory effect on OATP2B1 than do orange juice and GFJ. Tamai and coworkers recently conducted an in vitro uptake study in Xenopus oocytes expressing OATP2B1 and evaluated the inhibitory effect of GFJ, orange juice, and apple juice on the uptake of estrone-3-sulfate, a typical OATP2B1 probe drug.34 They found that coincubation with apple juice had a weaker inhibitory effect on OATP2B1, with the inhibition potency order of GFJ > orange juice > apple juice.34 The same trend was also obtained by another research group using estrone-3-sulfate and glibenclamide as OATP2B1 probe drugs.17 Because there is an in vitro and in vivo disconnect regarding the potency of different fruit juices on OATP2B1 inhibition, further investigation was conducted. A recent in vitro study evaluated the coincubation effect as before, but also evaluated the effect of preincubation with fruit juices, including GFJ, orange juice, and apple juice, on OATP2B1-mediated drug transport.33 The results showed that when OATP2B1-expressing Xenopus oocytes were preincubated for 15 minutes with different fruit juices, apple juice induced a remarkable decrease in OATP-mediated uptake of estrone-3-sulfate and fexofenadine. A similar but less potent effect was observed with orange juice, whereas GFJ did not exhibit the preincubation effect.33 Apple juice had the most potent preincubation effect at 15 minutes, and its inhibitory action lasted for at least 240 minutes.33 Because the order of potency of preincubation effect on OATP2B1 among these 3 fruit juices is consistent with the order of potency of the OATP2B1 inhibitory effect observed in vivo, the preincubation effect may play a bigger role than the coincubation effect for apple juice and orange juice for interactions with OATP2B1. In addition, the in vitro observation that GFJ has no preincubation effect is consistent with the clinical phenomenon that GFJ inhibition on OATP dissipates over a shorter period. Based on the long-lasting inhibitory effect of apple juice and orange juice on OATP2B1 observed in vitro, it is reasonable to speculate that these 2 fruit juices may have a prolonged effect on OATP2B1-mediated drug absorption in humans. Recently, Akamine et al. evaluated the effect of onetime apple juice ingestion on fexofenadine pharmacokinetics in healthy subjects, and they found that onetime ingestion of apple juice significantly decreased the AUC for (R)- and (S)-fexofenadine by

![Figure 3](image-url)
49% and 59%, respectively. This suggests that the OATP2B1 inhibition effect does not require repeated ingestion or a large volume of apple juice. The relationship between time of intake of apple and orange juices and their effect on OATP2B1-mediated drug disposition has not yet been evaluated in vivo. In addition, the exact mechanism of the preincubation effect of apple and orange juices on OATP2B1 remains unclear and represents another interesting topic that warrants further investigation.

In addition to fexofenadine, apple juice and orange juice have also been reported to substantially reduce the systemic exposures of many other OATP2B1 substrates. For example, orange juice and apple juice markedly reduced aliskiren peak plasma concentrations by 80% and 85%, respectively, and aliskiren AUC by 62% and 65%, respectively, in 12 healthy volunteers; there was no effect on aliskiren elimination half-life or renal clearance. Jeon et al. reported that apple juice greatly reduced atenolol exposures in healthy volunteers, and the effect was volume dependent: the 600- and 1200-mL volumes of apple juice diminished atenolol AUC to 42.0% and 18.5%, respectively, of that observed with water. In 1 clinical study that was conducted to evaluate the effect of orange juice on celiprolol disposition, celiprolol C\textsubscript{max} and AUC were decreased by 89% and 83%, respectively, when celiprolol was taken together with orange juice.

Although GFJ, orange juice, and apple juice administration has significantly reduced the systemic exposure of many OATP substrates, including fexofenadine, talinolol, celiprolol, and atenolol, an effect was not shown for several other drugs known to be transported by OATP. For example, GFJ did not affect the disposition of pravastatin and pitavastatin, although both are substrates of OATP2B1. One explanation for these negative interactions is that OATP may not play a major role in their absorption. Another possible explanation is that the effect of fruit juices on OATP is substrate- and inhibitor dependent. One potential mechanism for this substrate- and modulator-specific effect is the presence of multiple binding sites in the OATP2B1 protein that show distinct functionality. Tamai and colleagues found that OATP2B2 showed biphasic kinetics with high-affinity and low-affinity sites in transporting estrone-3-sulfate, and the uptake was pH dependent. The \( K_{m} \) values were 0.1 and 29.9 \( \mu \text{M} \), and the \( V_{\text{max}} \) values were 14.1 and 995 fmol/min per oocyte for the high-affinity and low-affinity sites, respectively. They also evaluated the effect of 12 compounds on estrone-3-sulfate uptake, and the results revealed that 4 compounds were inhibitors for both affinity sites, 4 compounds only inhibited the high-affinity site, 2 compounds only inhibited the low-affinity site, and 2 compounds had no effect on either site. These results indicated that there are at least 2 active sites on OATP2B1 that differ in substrate and inhibitor selectivity. Accordingly, the OATP2B1-mediated fruit juice–drug interaction would occur only when fruit juices and drugs share the same binding site on OATP2B1. Further evidence supporting the multiple-binding-site hypothesis was provided by Tamai’s group. In their study, they evaluated GFJ–drug interactions using OATP2B1-expressing Xenopus oocytes with 2 model drugs: fexofenadine, which interacts clinically with GFJ, and pravastatin, which does not. Both pravastatin and fexofenadine exhibited biphasic saturation kinetics, indicating the presence of multiple binding sites on OATP2B1. In addition, their results showed that GFJ strongly inhibited fexofenadine uptake mediated by the low-affinity site, but not pravastatin uptake by the low-affinity site. On the other hand, the uptake of pravastatin but not fexofenadine at the high-affinity site was strongly inhibited by GFJ. As both fexofenadine and pravastatin at clinically relevant concentrations are mainly transported via the low-affinity site on OATP2B1, only fexofenadine is likely to interact with GFJ on OATP2B1 at therapeutic concentrations. These data further support the concept of substrate dependence in OATP2B1-mediated fruit juice–drug interactions. This is important because the presence of multiple binding sites on OATP2B1 may at least partly explain observed differences in interactions based on binding-site affinities for substrate compounds. Regarding the effect of orange juice on OATP, another potential mechanism is the pH-lowering effect of orange juice. The uptake of OATP2B1 has been reported to be pH dependent. Kobayashi et al. reported that uptake of \([\text{3H}]\) estrone-3-sulfate and \([\text{14C}]\) pravastatin by OATP2B1 at pH 5.5 was significantly higher than that at pH 7.4. Koitabashi reported that orange juice increased the bioavailability of pravastatin in both rats and healthy human subjects. This may be because of the pH-lowering effect of orange juice, which subsequently increased OATP-mediated uptake of pravastatin.

**Green Tea**

The interaction between green tea catechins and OATPs has also been investigated. Roth et al. measured the uptake of the model substrate estrone-3-sulfate by cells expressing OATP1A2, OATP1B1, OATP1B3, or OATP2B1 in the absence and presence of the 4 most abundant catechins found in green tea, namely, EC, ECG, epigallocatechin (EGC), and EGCG. They found that EC and EGC did not significantly affect estrone-3-sulfate uptake by any of the 4 cell lines, indicating that they do not have a modulatory effect on the tested OATPs. On the other hand, ECG and EGCG significantly inhibited the uptake of estrone-3-sulfate uptake mediated by OATP1A2, OATP1B1, and OATP2B1. In addition, the authors observed the substrate-dependent effect of ECG and EGCG on OATP1B3-mediated uptake. These results indicated that 2 of the major flavonols present in...
green tea have a substantial effect on the function of OATPs expressed in the intestine and liver, which can potentially alter the disposition of coadministered OATP substrate compounds. Interestingly, ECG and EGCG were not only found to be modulators but also substrates of OATP, and both of them are transported by OATP1A2 and OATP1B3.25 In addition, the authors reported that the IC50 values of ECG and EGCG on OATP2B1 inhibition were estimated to be 35.9 and 101 μM, respectively.25 Notably, the average concentration of ECG and EGCG in brewed green tea is reported to be 450 μM (ie, 19.7 mg/100 mL) and 430 μM (ie, 77.8 mg/100 mL), respectively, with the maximal concentration of each catechin in the low millimolar range. Therefore, drinking a few cups of tea would result in the intestinal concentration of ECG and EGCG in the same range of their IC50 values, considering a gastric fluid volume of 100 to 500 mL on an empty stomach. Thus, the OATP-mediated green tea–drug interaction has the potential to be clinically important. The effect of green tea on the disposition of OATP substrates has been evaluated in humans. In a clinical trial conducted in Japan by Misaka and coworkers,144 10 healthy volunteers received a single oral dose of nadolol with green tea or water after repeated consumption of green tea with exceptionally high catechin content (700 mL/day) or water for 14 days. As shown in Figure 4, they found that green tea markedly decreased the Cmax and AUC of nadolol by 85.3% and 85%, respectively. In addition, the effects of nadolol on systolic blood pressure were significantly reduced by green tea. As nadolol undergoes minimal metabolism and has been reported to be an OATP1A2 substrate, the authors suggested that the interaction may be caused by the inhibitory effect of green tea catechins on OATP1A2. It should be noted that the green tea–type beverage used in this study is classed as “Food for Specified Health Use” by the Ministry of Health, Labour, and Welfare of Japan. It contains an exceptionally high total catechin content (~540 mg/bottle), and the catechin concentration is 2 to 5 times higher than that of typical bottled green tea sold in Japan145 and 6 to 50 times higher than that of typical bottled green tea in the United States. Further investigations, including studies that reflect actual green tea consumption patterns, are warranted to provide additional insight for assessing the potential risks associated with consuming green tea along with taking selected medications.

**Candidate Ingredients for OATP Inhibition**

The candidate active components in GFJ, orange juice, and apple juice responsible for OATP-mediated drug interactions have been evaluated recently in a number of in vitro and clinical studies.33,34,146 Naringin, the most abundant flavonoid present in GFJ, demonstrated a potent in vitro inhibitory effect on OATP1A2, with an IC50 value of 3.6 μM.146 Recently, naringin was also found to have a potent inhibitory effect on OATP2B1 (IC50 = 4.63 μM).34 Consistent with the potent inhibitory effect observed in vitro, Bailey et al. found that an

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Plasma concentrations of nadolol (circles) and systolic blood pressure (triangles) after oral administration of 30 mg nadolol with 700 mL of green tea or water following pretreatment with green tea or water for 14 days in 10 healthy volunteers. Adapted from Misaka et al. *Clin Pharmacol Ther.* 2014;95:432–438.
aqueous solution of naringin at the same concentration in GFJ (1200 μM) significantly reduced the exposure of fexofenadine in healthy volunteers, indicating that naringin is a major active ingredient in GFJ for OATP inhibition. As in the above study naringin was responsible for about half the reduction in the fexofenadine AUC compared with that seen with GFJ, other ingredient(s) in addition to naringin may also be responsible for the OATP inhibition by GFJ on fexofenadine absorption. In addition to flavonoids, furanocoumarins in GFJ, including bergamottin and 6',7'-dihydroxybergamottin, have also been evaluated, and no inhibitory effect on OATP was observed. Recently Tamai’s group screened a panel of ingredients in GFJ, orange juice, and apple juice to evaluate their effect on OATP. They reported that 8 major flavonoids (naringin, naringenin, hesperidin, hesperetin, phloridzin, phloretin, quercetin, and kaempferol) contained in the juices inhibited OATP2B1-mediated estrone-3-sulfate uptake with IC50 values of 4.63, 49.2, 1.92, 67.6, 23.2, 1.31, 9.47, and 21.3 μM, respectively. Based on the potency of OATP2B1 inhibition and typical amounts found in fruits juices, naringin and hesperidin are reported to be the major OATP2B1 inhibitors in GFJ and orange juice, respectively, whereas a combination of multiple components appears to be responsible for OATP2B1 inhibition by apple juice. It should be noted that these flavonoids were all evaluated under coincubation conditions. As noted earlier, apple juice has a weak inhibitory effect on OATP2B1 in vitro under coincubation conditions, but it demonstrated a long-lasting inhibitory effect under preincubation conditions. The specific ingredient(s) responsible for the long-lasting inhibitory effect of apple juice on OATP2B1 are unknown and warrant further investigation. Regarding green tea, the information related to its candidate active ingredients on OATP inhibition is provided in the Green Tea section above.

**Beverage–Drug Interactions Mediated by Other Enzymes/Transporters**

The primary focus of beverage–drug interaction research has been on CYP3A, P-gp, and OATP. There is limited information on beverage–drug interactions mediated by other enzymes and transporters. GFJ has been reported to inhibit the phase II enzyme sulfotransferase (SULT) 1A1 and SULT 1A3. In addition, drugs that are substrates of esterase enzymes were affected in vitro by GFJ. The clinically relevant beverage–drug interactions mediated by intestinal OATP stimulated research on the effect of fruit juices on other intestinal uptake transporters. For example, Minmura et al. evaluated the in vitro effect of atenolol on human organic cation transporter 1 (OCT1), an uptake transporter expressed at the brush border membrane of enterocytes. They reported that OCT1-mediated atenolol transport can be inhibited by various flavonoids, including those that are contained in fruit juices. Similarly, Staub et al. investigated the effect of fruit juices on peptide transporter 1 (PEPT1), an important uptake transporter that is also expressed in the apical side of intestine, with glycyrsarcosine and cefadroxil being used as the substrates. They found that PEPT1-mediated drug uptake was decreased when they were coincubated with fruit juices, but the in vitro effect on PEPT1 was less than that on OATP. We recently reported that ingredients in fruit juices, especially those in orange juice, have potent inhibitory effect on the efflux transporter breast cancer resistance protein (BCRP). Among the 14 ingredients screened, 4 ingredients in GFJ (ie, bergamottin, 6',7'-dihydroxybergamottin, quercetin, and kaempferol), 2 ingredients in orange juice (tangeretin and nobiletin), and 1 ingredient in apple juice (ie, hesperetin) greatly inhibited BCRP-mediated dasatinib efflux at a concentration of 50 μM (P < .001). Further concentration-dependent studies revealed that bergamottin, 6',7'-dihydroxybergamottin, tangeretin, and nobiletin are potent BCRP inhibitors, with IC50 values of 3.19, 5.2, 1.19, and 1.04 μM, respectively. It should be noted that the information in this section is from in vitro experiments. The potential effect of beverage on these enzymes/transporters in humans has not been established, and further in vivo and clinical investigations are warranted.

**Conclusion and Future Directions**

Since the initial finding in 1989 that GFJ significantly increased felodipine blood concentrations, beverage–drug interaction remains an area of active research and has received extensive investigations in the past 2 decades. The known mechanisms for clinically relevant beverage–drug interactions include CYP3A and OATP (Figure 3). For CYP3A-mediated beverage–drug interactions, the in vivo CYP3A inhibitory effect is limited to a few fruit juices, including GFJ, Seville (sour) orange juice, and pomelo grapefruit juice. It should be noted that, although numerous CYP3A substrates have been evaluated, a strong degree of interaction (ie, AUC ratio ≥ 5) with GFJ only occurs with a fairly small number of drugs. This is not surprising, as GFJ only has an inhibitory effect on enteric CYP3A. As a result, significant CYP3A-mediated GFJ–drug interaction only occurs when a CYP3A substrate is orally administered and undergoes extensive first-pass metabolism via enteric CYP3A. It should be noted that the magnitude of pharmacokinetic interaction should not be the only factor to evaluate the potential risk of GFJ–drug interaction. For those drugs with a narrow therapeutic window (such as tacrolimus) known to interact with GFJ and for elderly people, who are less tolerant of the fluctuation in drug exposure, GFJ should be avoided even if the magnitude of interaction on the pharmacokinetic side is minimal or mild. In addition,
purity and amount of the constituents in these beverages may be variable and inconsistent, which represent potential confounding factors in interpreting the data in these studies and in applying it clinically. The furanocoumarins in GFJ are responsible for the clinically significant CYP3A inhibition. Several methods, including filtration, heating, and UV radiation, have been developed to remove the furanocoumarins in GFJ. Recently, GFJ cultivars with extremely low levels or free of furanocoumarins have been developed through genetic modification, which may have the potential to be adapted for large-scale production. Although CYP3A-mediated beverage–drug interactions are limited to GFJ, clinically significant OATP-mediated beverage–drug interaction have been observed with not only GFJ, but also orange juice, apple juice, and, most recently, green tea. In contrast to CYP3A, OATP inhibition by beverages appears to be competitive and short-lived. In addition, the extent of the interaction is influenced by the volume of the juice/beverage consumed. This suggests that the magnitude of the interaction could be minimized by reduced consumption or be avoided by separating drug administration from beverage ingestion.

Among the clinical studies that demonstrated clinically significant beverage–drug interactions, many of these were carried out in conditions in which subjects received double-strength and/or unrealistically large quantities of beverages, neither of which represents typical consumer usage. As a result, the effect of beverages observed may not realistically reflect the real magnitude of interaction in the way in which patients ordinarily take medication. An example is the green tea study, in which a product with exceptionally high total catechin content was found to markedly decrease nadolol exposure in healthy volunteers (Figure 4). As the catechin concentration ingested in that study was 2 to 5 times higher than that of typical bottled green tea in Japan and 6 to 50 times higher than that of typical bottled green tea in the United States, it is difficult to translate the applicability of the findings to a real-world setting. Further investigations, including studies that reflect actual fruit juice/green tea consumption patterns, are warranted to provide additional insight into assessing the potential risks associated with consuming these beverages. As clinically relevant beverage–drug interactions only occur in a fairly small number of CYP3A and/or OATP substrates and most of the interaction are only modest, complete avoidance of these beverages is unwarranted for the majority of patients. However, as noted earlier, for patients who take narrow therapeutic range drugs such as tacrolimus. Furthermore, in those cases in which clinically relevant beverage–drug interactions are possible, therapeutic alternatives are usually available to avoid the interaction.

Declaration of Conflicting Interests
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References


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