The effects of fruit juices on drug disposition: a new model for drug interactions

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Abstract
Grapefruit juice produces mechanism-based inhibition of intestinal drug metabolism when consumed in normal quantities. This can produce clinically important increases in oral drug bioavailability when coadministered with substrates of cytochrome P450 3A4 (CYP3A4) that undergo high presystemic metabolism. Furanocoumarins such as bergamottin and 6′,7′-dihydroxybergamottin have been identified as probable active constituents. Grapefruit juice may also inhibit intestinal P-glycoprotein-mediated efflux transport of drugs such as cyclosporine to increase its oral bioavailability. However, grapefruit juice does not enhance the absorption of digoxin, a prototypical P-glycoprotein substrate, likely because it has high inherent oral bioavailability. Grapefruit and other fruit juices have recently been shown to be potent in vitro inhibitors of a number of organic anion-transporting polypeptides (OATPs). These juices were also found to decrease the absorption of the nonmetabolized OATP substrate, fexofenadine. Taken together, the data support inhibition of intestinal uptake transporters by fruit juices to decrease drug bioavailability. This would represent a new mechanism for food–drug interactions. These findings with grapefruit and other fruit juices continue to enhance our understanding of the complex nature of food–drug interactions, and their possible influence on the clinical effects of medications.

Keywords Cytochrome P450, drug interactions, fruit juice, grapefruit juice, organic anion-transporting polypeptides, P-glycoprotein.

Introduction
Medications and food are often taken together. Linking drug administration to a regular event like a meal can improve adherence of the patient to a treatment regimen, especially in the elderly [1]. However, concomitant drug and food intake creates the opportunity for interactions that may change the oral bioavailability and resulting effectiveness or toxicity of a drug.

Oral bioavailability is dependent upon several factors that include rate and extent of drug dissolution from the solid dose form and transfer of drug from the stomach to the duodenum where it is normally absorbed. A drug must also pass through the gut wall, enter the portal circulation and pass through the liver before gaining access to the systemic circulation. Both the gut wall and the liver are capable of affecting oral drug bioavailability by mechanisms involving presystemic metabolism and transport of drugs [2]. More than 10 years ago, our group was the first to report the effect of a citrus juice on oral drug bioavailability [3]. Grapefruit juice was shown to increase the oral bioavailability and effects, both adverse and advantageous, of the dihydropyridine calcium channel antagonists, felodipine and nifedipine. Subsequently, grapefruit juice has been found to interact with more than 25 medications from a wide range of therapeutic categories [4–7]. Drugs showing enhanced oral bioavailability and those demonstrating no change or a decrease in oral bioavailability with grapefruit juice are listed in Tables 1 and 2, respectively. This paper will discuss the effects of grapefruit juice and other fruit juices on mechanisms that affect oral drug bioavailability: first-pass metabolism, intestinal efflux and uptake transport.

Effect on first-pass drug metabolism
Pharmacokinetics
Felodipine has been the most extensively studied probe for grapefruit juice-drug interactions since the initial observation. Normally, felodipine is completely absorbed from
Table 1 Drugs demonstrating increased oral bioavailability with grapefruit juice

<table>
<thead>
<tr>
<th>Anti-infective agents</th>
<th>Anticonvulsants</th>
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<tr>
<td>artemether</td>
<td>buspirone</td>
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<td>bendazoles</td>
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<td>erythromycin</td>
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<td>praziquantel</td>
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<td>saquinavir</td>
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<td>simvastatin</td>
<td>Histamine H1 antagonants</td>
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<td>Cardiovascular agents</td>
<td>Gastrointestinal agents</td>
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<td>amiodarone</td>
<td>astemizole</td>
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<td>carvedilol</td>
<td>terfenadine</td>
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<td>felodipine</td>
<td>cyclosporine</td>
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<td>nicardipine</td>
<td>tacrolimus</td>
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<td>nifedipine</td>
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<td>sildenafil</td>
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<td>verapamil</td>
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The structural modification of CYP3A4 would be expected to inactivate the enzyme, which may explain the prolonged effect of grapefruit juice [17].

Mechanism of inhibition

The decreased intestinal CYP3A4 protein levels observed after grapefruit juice consumption implied that the interaction was not simple competitive inhibition. The content of enterocyte CYP3A4 mRNA was not changed, thus it appears that grapefruit juice did not decrease CYP3A4 protein content by reducing its transcription [16]. Rather, it indicates that the inhibitory effect results from reduced translation, or enhanced degradation of the CYP3A4 protein. This effect would be consistent with mechanism-based or ‘suicide’ inhibition in which substances in grapefruit juice covalently bond to the enzyme to inactivate it [6]. The structurally modified CYP3A4 would be expected to undergo rapid proteolysis within the cell. Consequently, the return of CYP3A4 activity would require de novo enzyme synthesis, which may explain the prolonged effect of grapefruit juice [17].
Clinical relevance

These findings illustrate several important concepts. They show that the interaction with grapefruit juice is relevant only for orally administered drugs. Importantly, they demonstrate the significance of the gut as a site for drug metabolism. They suggest that drugs with high inherent presystemic metabolism are at risk of producing interactions of this type. Because a normal amount of grapefruit juice consumed 24 h preceding drug therapy has been shown to augment bioavailability [17], grapefruit juice consumption should be avoided entirely during pharmacotherapy.

Active constituents

Since 1991, considerable effort has been directed at determining the constituents of grapefruit juice that increase oral drug bioavailability. As a food, grapefruit juice is composed of many structurally diverse substances, and although the active ingredient in grapefruit juice has not been definitely identified, several substances have been proposed on the basis of results from in vitro studies of inhibition of CYP3A4 activity. Prior to the discovery of the effect of grapefruit juice, flavonoids had been identified as inhibitors of cytochrome P450 enzymes [18]. Early attention focused on the flavonoid, naringin, which is present in high concentrations in grapefruit juice, and its more active aglycone metabolite, naringenin, as potentially active constituents [19,20]. Both of these flavonoids are competitive inhibitors of CYP3A4. However, when pure naringin was tested in humans at a concentration approximating that in grapefruit juice, it did not produce a metabolic interaction with felodipine or nisoldipine [21,22]. Thus, naringin by itself does not appear to be an important clinical inhibitor of CYP3A4 activity.

Other proposed active ingredients include the furanocoumarins, bergamottin, 6′-epoxybergamottin, 6′,7′-dihydroxybergamottin and related dimers [23–27]. Seville (sour) orange was found to contain bergamottin and 6′,7′-dihydroxybergamottin as well as naringin [28]. Consequently, the effect of Seville orange juice on the prototypical CYP3A4 substrate, felodipine, was determined in human volunteers [28]. Felodipine bioavailability was 1·8-fold and 1·9-fold that of control values, respectively, for Seville orange juice and grapefruit juice (diluted to contain equivalent total bergamottin plus 6′,7′-dihydroxybergamottin concentrations). They were compared with orange juice that contains none of these substances as a control. The pharmacokinetics of the dehydrofelodipine metabolite with Seville orange juice and grapefruit juices supported inhibition of CYP3A4 activity. Thus, both bergamottin and 6′,7′-dihydroxybergamottin appear to be clinically important inhibitors of CYP3A4 in these juices, although other furanocoumarins and naringin may also be involved.

This study was important for an additional reason. A previous report had shown that Seville orange juice did not augment plasma concentrations of the CYP3A4 substrate, cyclosporine, but grapefruit juice increased its bioavailability to 1·6-fold that of control (water) [29]. This lack of effect by Seville orange juice occurred despite a marked decrease in enterocyte CYP3A4 content. Therefore, inhibition of intestinal CYP3A4 cannot be the only mechanism whereby grapefruit juice enhances the oral bioavailability of cyclosporine. Since cyclosporine disposition is determined not only by CYP3A4, but also by P-glycoprotein [30], these data support the concept that grapefruit juice and possibly other fruit juices may alter oral drug bioavailability by affecting P-glycoprotein activity.

Effect on P-glycoprotein

Transporters are increasingly recognized as important determinants of drug disposition and resulting clinical response. The best-characterized drug transporter is P-glycoprotein. It transports numerous structurally and therapeutically unrelated drugs including cyclosporine, digoxin, erythromycin, lovastatin, loperamide, HIV-protease inhibitors and many chemotherapeutic agents [31]. P-glycoprotein is located on the luminal surface of epithelial cells of the small intestine, the bile canalicular membrane of the liver and the proximal tubule of the kidney [32]. It is also present in endothelial cells that comprise the blood–brain, blood–testes, and maternal–fetal placental barriers [33,34]. P-glycoprotein affects the disposition of drugs by limiting their absorption from the gut, by facilitating their removal by secretion into bile and urine and by reducing their entry into brain, testes and placenta.

In addition to inhibition, P-glycoprotein activity can be augmented. Increased activity of P-glycoprotein has been reported secondary to positive cooperativity at two or three binding sites [35,36] and secondary to induction of protein expression. Apart from one in vitro study that suggested increased activity with grapefruit juice [37], all other in vitro studies of the effect of grapefruit juice on P-glycoprotein have reported inhibition of function, using various drug substrates [38–44]. The evidence to date therefore suggests that positive cooperativity does not occur with grapefruit juice or its constituents. Changes in P-glycoprotein levels in the human gastrointestinal tract were evaluated following ingestion of 8 oz of grapefruit juice consumed three times per day for 5 days [16]. In this study, there was no significant change in P-glycoprotein levels from baseline, suggesting that grapefruit juice neither induces nor causes proteolysis of this protein.

Investigations of the effect of grapefruit juice on P-glycoprotein activity require the use of probe drugs that are only substrates for P-glycoprotein efflux transport. Unfortunately, it has been difficult to identify such probe drugs for this purpose. Many drugs that are substrates for P-glycoprotein are also metabolized by CYP3A4, so that changes in presystemic elimination as a result of grapefruit juice administration may be secondary to either changes in P-glycoprotein activity, or CYP3A4 activity [45,46]. A limited number of interaction studies with grapefruit juice have been conducted with drugs that are substrates for
P-glycoprotein but not CYP3A4 in order to eliminate possible confounding results by effects on this enzyme.

**Digoxin**

Digoxin is a well-characterized P-glycoprotein substrate with negligible metabolism in humans [47,48]. The effect of 220 mL single strength grapefruit juice was compared with water consumed 30 min before and 3·5, 7·5, and 11·5 h after 0·5 mg of orally administered digoxin in 12 healthy volunteers [49]. With grapefruit juice, mean digoxin AUC$_{0-24}$ was minimally increased to 1·1-fold that with water (not statistically significant). Unfortunately, P-glycoprotein does not appear to limit the oral absorption of digoxin, since this drug has inherently good oral bioavailability of 70%–80% [50]. Consequently, complete inhibition of P-glycoprotein would be expected to increase digoxin bioavailability to 1·2–1·3-fold that with water. Therefore, the minimal effect in this study may be a function of the high inherent oral bioavailability of digoxin and should not be interpreted as evidence for lack of effect of grapefruit juice on P-glycoprotein activity in humans.

Several studies have examined the effect of both whole grapefruit juice and its constituents on P-glycoprotein activity *in vitro*, using both digoxin and other probe drugs as substrates [38–40,42–44]. Unfortunately, differing experimental models, substrate drugs and inhibitory conditions have produced conflicting results that preclude adequate synthesis of this information.

**Fexofenadine**

The P-glycoprotein substrate, fexofenadine, has an absolute oral bioavailability estimated at 33% in humans (Hoechst Marion Roussel, product information). It is eliminated from the body unchanged mainly by biliary and gastrointestinal secretion in faeces, with a much smaller amount excreted in urine [51]. If it is assumed that P-glycoprotein-mediated drug efflux is the rate-limiting step in the absorption of fexofenadine from the gastrointestinal tract, and it is assumed that grapefruit juice is an inhibitor of this process, then it would be concluded that with coadministration of grapefruit juice, bioavailability of fexofenadine should be increased. However, grapefruit juice markedly decreased, rather than increased, plasma fexofenadine AUCs and C$_{max}$ [38]. This was associated with no change in fexofenadine $t_{max}$ and $t_{1/2}$ consistent with reduced bioavailability as opposed to enhanced systemic elimination. There was reduced renal fexofenadine excretion and lack of change in renal fexofenadine clearance, which further supports this conclusion. These surprising effects were also observed with orange juice and apple juice. Given that there is no evidence that grapefruit juice can induce or activate P-glycoprotein, an alternate explanation was required. Fexofenadine is also a substrate for the organic anion-transporting polypeptides (OATPs) [52]. The OATPs were initially not believed to contribute to the bioavailability of fexofenadine, but given our findings we hypothesized that the interaction observed between these fruit juices and fexofenadine results from inhibition of activity of OATPs, at least in part. These proposed effects of fruit juices are illustrated in Fig. 1.

**Effect on OATPs**

Uptake and efflux transporters can be expressed in the same cell [31]. In the intestine, both OATP and P-glycoprotein transporters are located on the luminal membrane of the enterocyte, which results in opposing vectors for uptake into the portal circulation and for efflux back into the bowel [53] (see Fig. 1). In the liver, the location of OATPs on the basolateral membrane and P-glycoprotein on the bile canaliculus membrane of hepatocytes facilitates the efficient unidirectional movement of drug from portal circulation into bile for secretion back into the intestine [54–57].

In *vitro* studies using grapefruit, orange and apple juices and their constituents as modulators of OATP-mediated fexofenadine uptake were pursued [38]. Grapefruit juice potently inhibited OATP-mediated fexofenadine uptake by human and rat transporters in a dose-dependent fashion. Grapefruit juice concentration at only 0·5% normal strength
decreased the activity by half. This contrasted with the effect of grapefruit juice at 10-fold higher concentration, which did not alter P-glycoprotein efflux activity. Orange juice produced nearly identical results as grapefruit juice. Apple juice also reduced activity of OATP transporters but it appeared to differ in the spectrum of uptake transporters affected. Thus, several fruit juices produced relatively more potent inhibition of several OATP transporters than P-glycoprotein.

In vivo, fexofenadine AUCs under baseline conditions varied among individuals [38]. It might be expected that those subjects with high fexofenadine AUC would have relatively high intestinal OATP activity. There was a high correlation between baseline fexofenadine AUC and magnitude of decrease with juice among individuals. Since elevated enzyme or transporter activity is generally more sensitive to inhibition, these findings suggest that grapefruit, orange and apple juices acted by preferentially reducing intestinal OATP function.

Although a direct inhibitory effect by fruit juices on intestinal OATP transporters may explain the interaction observed in humans, other mechanisms need to be considered. If water were absorbed from the gut faster when administered alone than in fruit juice, this may produce higher fexofenadine concentration in intestinal fluid and slower gastrointestinal transit time. This could cause higher drug exposure to OATP transporters and result in greater drug absorption. Thus, another mechanism for decreased fexofenadine bioavailability by fruit juices could involve an indirect effect on OATP function from enhanced intestinal fluid volume by nonspecific osmotic effects of solutes. In an attempt to assess the possible importance of direct inhibition of OATP function in humans, a pilot project was conducted [38]. Grapefruit juice was processed by solid phase column chromatography to obtain a nonpolar fraction that contained specific constituents shown to inhibit in vitro OATP activity, but not substances that contributed to the majority of the osmotic property of the juice. The effect on fexofenadine bioavailability by 300 mL grapefruit juice, water and nonpolar fraction reconstituted in water was compared. This volume of grapefruit juice produced lower plasma fexofenadine concentrations than those with water. The nonpolar fraction also caused lower fexofenadine concentrations compared with water. Thus, direct inhibition by specific constituents in grapefruit, and possibly other juices, may be a mechanism explaining at least part of the interaction.

The results of the interaction study between fruit juices and fexofenadine expands our understanding of the possible interplay between transporters that determine drug disposition. This model now includes the role of intestinal drug uptake by OATP transporters in addition to drug efflux transport by P-glycoprotein. It appears that fruit juices and constituents can preferentially inhibit members of the OATP transporter family. The clinical consequence of such an interaction is a significant reduction in the oral bioavailability of fexofenadine and possibly other drugs. The result of this study suggests a new mechanism to account for certain food–drug interactions.

Conclusions

Metabolism, efflux transport and uptake transport in the intestine and the liver can each contribute to the disposition of drugs. Foods have been shown to alter drug disposition. Grapefruit juice can cause mechanism-based inhibition of intestinal CYP3A4 in humans, and may also inhibit drug uptake and efflux transport activities. In this paper, we have reviewed the first evidence that fruit juices can alter intestinal drug uptake transport. Many questions remain to be answered. Of particular interest are the specific substance(s) responsible for the inhibitory effects in grapefruit, apple and orange juices. Unlike the metabolic effects of grapefruit juice, the relevance of the inhibitory effect of grapefruit juice on drug uptake transport has yet to be established across a range of substrate drugs. However, it is becoming increasingly apparent that constituents from a number of foods can act as specific modulators of important processes that determine drug disposition and resulting clinical response.

References


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