Expert Opinion

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Influence of fruit juices on drug disposition: discrepancies between *in vitro* and clinical studies

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Background: Grapefruit juice is known to alter the pharmacokinetics of over 30 prescription drugs by increasing their bioavailabilities. After the discovery of this interaction almost 20 years ago, there have been many reports investigating the effects of fruit juices on drug disposition. *Objective*: This article reviews the literature on fruit juice–prescription drug interaction studies to determine which juices are likely to cause clinically significant interactions. *Methods*: We examined the results from *in vitro* and clinical studies regarding the interactions between prescription drugs and over ten fruit beverages. *Results/conclusions*: Grapefruit juice and Seville orange juice caused several clinically significant interactions with cytochrome P4503A (CYP3A). The OATP drug transporter was inhibited by grapefruit juice, orange juice, and apple juice. Other fruit juices also interacted with drug metabolizing enzymes and transporters *in vitro*, but more studies are needed to determine whether these interactions are clinically significant.

Keywords: 6′7′-dihydroxybergamottin, cranberry, furanocoumarins, grapefruit, orange, pomegranate

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1. Introduction

In 1989, Bailey *et al.* [1] accidentally discovered an interaction between grapefruit juice (GFJ) and the calcium-channel blocker felodipine, characterized by a substantial increase in the systemic exposure (AUC) of the drug due to co-administration with GFJ. Two years later, in 1991, they reported that grapefruit juice also interacted with another calcium channel blocker, nifedipine [2]. As a result of co-administration with grapefruit juice, the AUC of felodipine and nifedipine increased by 184% and 34%, respectively. Since this initial report, over 30 drugs were found to be affected by grapefruit juice [3]. Other fruit juices, such as orange, cranberry, grape and pomegranate, have also been studied regarding their influence on drug disposition.

The possibility of an interaction between fruit juices and prescription drugs creates a dilemma for individuals who consume these juices for their health benefits. Flavonoids and other polyphenolic compounds contained in fruit juices have been associated with a decrease in the incidence of cardiovascular disease [4,5], some types of cancer [6,7], and possibly the augmentation of HIV treatment regimens [8].

This article reviews the most current literature on interactions between prescription drugs and fruit juices. As will be shown, fruit juices are more likely to inhibit drug metabolism *in vitro* than in humans. The reason for this discrepancy is not entirely understood, but it is a phenomenon that has been

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Table 1. Concentrations of naringin, bergamottin, and 6′,7′-dihydroxybergamottin in three different types of grapefruit juice.

	Naringin	Bergamottin	DHB
Type of grapefruit juice (n)	Concentration (micromolar)		
Pink (3 brands)	782 ± 113	10.0 ± 2.9	0.6 ± 0.3
White (5 brands)	1010 ± 287	24.5 ± 7.6	14.5 ± 22.1
Red (6 brands)	473 ± 277	9.5 ± 6.3	5.6 ± 6.5

For each type of juice, 3 – 6 brands were tested. Means are shown as ± standard deviation.
Adapted from de Castro *et al.* [25].

observed with herbal products as well [9]. We summarize *in vitro* and *in vivo* interactions between prescription drugs and over ten different types of beverages including grapefruit juice, orange juice, Seville orange juice, pomelo juice, lemon juice, lime juice, tangerine juice, citrus soft drinks, cranberry juice, pomegranate juice, red wine, and grape juice.

In general, clinically important interactions are more likely to occur when the perpetrator is a significant modulator of a metabolic enzyme, or the therapeutic index of the drug is narrow. While only a few clinically significant juice–drug interactions have been observed, *in vitro* and animal studies suggest that fruit juices could influence the activities cytochrome P450 enzymes and drug transporters [10-13], and this evidence should be examined before administering fruit juices to patients on multiple drug regimens.

2. Influence of fruit juices on drug disposition

2.1 Grapefruit juice

Of all the fruit juices studied, GFJ has been reported to cause the greatest number of clinically significant interactions with prescription drugs. The mechanism of interaction is thought to be the inhibition of cytochrome P4503 A4 (CYP3A4) [14,15], and possibly inhibition of drug transporters, particularly the organic anion-transporting polypeptides (OATP) uptake transporter [16-18]. Inactivation of CYP3A4 occurs primarily in the intestine, and therefore only orally administered drugs with high presystemic extraction are affected [14]. The mechanism of CYP3A inhibition is irreversible, and complete recovery takes about three days after a 10-oz (300-ml) dose of juice [19]. For this reason, grapefruit juice can also interact with drugs taken many hours after the consumption of the juice [20]. The bioavailability of lovastatin was doubled when taken 12 h after [21], and the AUC of felodipine was still significantly affected when it was administered 24 h after consumption of grapefruit juice [22].

2.1.1 *Chemistry of grapefruit juice*

The primary constituents in GFJ responsible for the inhibition of CYP3A are believed to be naturally occurring furanacoumarins, particularly bergamottin and 6′,7′-dihydroxybergamottin (DHB) [23-25]. De Castro *et al.* [26] examined the concentrations of these constituents in pink, white, and red grapefruit juices (**Table 1**). While there was significant variation among different brands, bergamottin and DHB were found in the micromolar concentrations in most samples. Grapefruit juice also contains a number of furanocoumarin dimers, also known as spiroesters or Paradisins. These dimers may carry the designation as GF-I-1 and GF-I-4, and are found in relatively small quantities in grapefruit juice, but can inhibit CYP3A as strongly as ketoconazole [27,28]. Furanocoumarins can also inhibit the activities of other cytochrome P450 enzymes *in vitro*. Isolated furanocoumarins appear to inhibit most of the major cytochrome P450 enzymes: bergamottin can inhibit CYPs 1A2, 2C9, 2C19, 2D6 and 3A4, and DHB can inhibit CYPs 1A2 and 3A4 [29].

The role of furanacoumarins in the inhibition of CYP3A activity in humans was confirmed recently by Paine *et al.* [30]. In this study, furanacoumarin-free grapefruit juice (FC-free GFJ) was prepared using a citrus-debittering system, and administered to 18 healthy volunteers along with the CYP3A probe, felodipine. The negative control in this study was orange juice, which has been reported not to inhibit the metabolism of felodipine [2,3]. The results, which compared the pharmacokinetics of felodipine when co-administered with orange juice (OJ), GFJ, and FC-free GFJ, confirmed that furanocoumarins did cause the increase in bioavailability of the drug. The AUC for felodipine when administered with concentrated GFJ was twice as high as when it was administered with the other two juices. The pharmacokinetic profile of felodipine was similar when it was administered with either OJ or FC-free GFJ.

The mechanism by which furanocoumarins inhibit enzyme activity is not completely understood. Kinetic studies have suggested that the inhibition of CYP3A is mechanism-based, whereby the inhibitor binds to the enzyme, and irreversibly deactivates it. There is now also evidence that CYP3A4 is not the only enzyme affected by furanocoumarins. Lin *et al.* [31] have shown that bergamottin inactivates other cytochrome P450 enzymes, particularly CYPs 2B6 and 3A5. While CYP3A5 is expressed at lower levels than CYP3A4, it is still a significant enzyme from a drug metabolism point of view. First, this enzyme is prevalent among African Americans, and second, it is commonly found in children under 19 years of age [31,32]. The mechanism of inhibition of these two enzymes appears to occur through the destruction of the heme group and the covalent binding of a reactive intermediate of bergamottin to the apoprotein. It is important to keep in mind, however, that clinical studies frequently do not correlate with *in vitro* results. To our knowledge, there is no evidence for the inhibition of CYP3A5 or CYP2B6 by grapefruit juice in humans. Therefore, *in vitro* results should be used primarily as a guide for what needs to be tested in clinical studies.

Naringin, a flavonoid glycoside, and naringenin, a flavanone, are both found naturally in grapefruit juice, and have been reported to inhibit transport by P-glycoprotein *in vitro* [3,12,33,34], but there is not enough clinical data to determine whether whole grapefruit juice is a significant inhibitor of P-glycoprotein in humans. Naringin, found in micromolar concentrations in grapefruit juice (see **Table 1**), has also been shown to inhibit OATP1A2 activity [35] and, as will be shown below, this interaction appears to be clinically significant.

2.1.2 *In vitro studies*

2.1.2.1 *Drug metabolism*

Grapefruit juice and its constituents inhibit multiple cytochrome P450 enzymes *in vitro*. Tassaneeyakul *et al.* [36] tested the inhibitory potency of whole grapefruit juice, bergamottin, DHB, the dimers GF-I-1, GF-I-2, and GF-I-4, and a sesquirterpene, nootkatone, in human liver microsomes. CYP3A activity was inhibited by all grapefruit juice components, especially the furanocoumarins dimers GF-I-1 and GF-I-4. The activity of CYP3A was tested by measuring nifedipine oxidation, omeprazole sulfoxidation and omeprazole-3 hydroxylation. On average, the IC_{50}s in these reactions for bergamottin and DHB were between 250 nM and 1700 nM. As **Table 1** shows, bergamottin and DHB are found in micromolar quantities in grapefruit juice, suggesting that these interactions could be significant. The IC_{50}s for GF-I-1 and GF-I-4 were between 3 and 15 nM, suggesting much stronger inhibition by furanocoumarins dimers than by DHB or bergamottin.

Whole grapefruit juice extract inhibited CYP 1A2, 2C9, 2D6 and 3A4. Bergamottin, DHB, GF-I-1 and GF-I-4 also inhibited other major cytochrome P450 enzymes. The IC_{50}s for CYP 1A2, 2C9, 2C19 and 2D6 were between 100 and 5000 nM. Interestingly, CYP 2E1 was the least sensitive to inhibition. Nootkatone did not significantly inhibit enzyme activity, except for CYP2A6 and CYP2C19. The IC_{50}s for these reactions were approximately 11.5 and 22.5 nM, respectively.

2.1.2.2 *Transporter studies*

Grapefruit juice has been recognized for nearly 20 years as a significant inhibitor of CYP3A. The influence of GFJ over transporters, however, is only recently being recognized, and the results of transporter studies are conflicting. Grapefruit juice was reported to inhibit the transport of the P-glycoprotein substrates Rhodamine123, fexofenadine and saquinavir in rat intestines [37], and it also inhibited P-glycoprotein and MRP2 (multi-drug resistance protein) in Caco-2 cells [38]. On the other hand, GFJ activated the transport of vinblastine, cyclosporine, digoxin, fexofenadine and losartan in MDCK-MDR1 cells [39]. Therefore, the effect of GFJ on P-glycoprotein has not been clearly established.

GFJ does appear to influence the activity of the OATP influx transporter. In an *in vitro* study with transfected HeLa cells, the OATP-mediated uptake of fexofenadine was reduced by approximately 40% when the cells were exposed to 0.5% juice, and the transport was almost eliminated by 5% juice [12]. In the same study, P-glycoprotein-mediated digoxin or vinblastine transport were not significantly affected.

2.1.3 *Clinical studies with grapefruit juice*

2.1.3.1 *Drug metabolism*

The consumption of grapefruit juice has been shown to lead to clinically significant food–drug interactions, due to irreversible inhibition of CYP3A [6]. Some of the most significant interactions occured with simvastatin [40], lovastatin [41], and buspirone [42], where the AUC increased by factors of 16.1, 15.3, and 9.2, respectively. The accompanying fold changes in C_{max} were also considerable: 9.4, 11.8, and 4.2, respectively [3]. Grapefruit juice is also advised to be avoided when taking other drugs such as the antiarrhymic amiodarone, anxiolytics such as buspirone, diazepam and triazolam, as well as immunosuppressants such as cyclosporine and tacrolimus [43].

Inhibition of CYP3A occurs mainly in the intestine, therefore only drugs which are orally administered and undergo significant pre-systemic extraction by enteric CYP3A are affected [14]. The pharmacokinetics of cyclosporine, for example, were not affected in a clinical study when the drug was administered intravenously [44]. After oral administration, however, the AUC of the drug increased by nearly 40%, suggesting that only intestinal CYP3A was affected by grapefruit juice. There is evidence, however, that consumption of large quantities of grapefruit juice could affect hepatic CYP3A. In particular, the half-lives of triazolam [45,46] and midazolam [47] were prolonged after volunteers consumed concentrated grapefruit juice two or three times a day, suggesting that hepatic CYP3A was also inhibited. It is believed that grapefruit juice is a potent inhibitor of CYP3A *in vivo* because it binds irreversibly to the enzyme. As it was recently shown, the recovery half-life of CYP3A after 10 oz of regular strength grapefruit juice is about 23 h, and it can take up to 3 days to fully regenerate the enzyme [19,46].

The mechanism of CYP3A inhibition was recently investigated in humans through the examination of small bowel and colon mucosal biopsies [15,48]. Consumption of grapefruit juice for 5 days led to an over 60% reduction of intestinal CYP3A4 and CYP3A5 protein content. The CYP3A activity in the liver, and the intestinal CYP2D6 and CYP1A1 content, were not affected. In addition, the decrease in the intestinal CYP3A4 was detected just 4 h after the consumption of the juice. An interesting finding was that the CYP3A4 mRNA content in the small bowel was not changed, suggesting that the inhibition could occur through a post-transcriptional mechanism such as accelerated CYP3A4 degradation. Therefore, the recovery of CYP3A activity would need to occur through

de novo enzyme synthesis, explaining the long regeneration time of the enzyme [15,19].

Consumption of grapefruit juice is of particular concern for the elderly, who might be concurrently taking multiple medications. The administration of felodipine is of special concern, because its clearance is decreased in the elderly compared with younger individuals [49]. Furthermore, the elderly might be more susceptible to the drug's anti-hypertensive effects [50]. To date, there have been few studies which investigated the interactions between grapefruit juice and felodipine, but there is sufficient evidence to suggest that normal doses of grapefruit juice (250 ml) can cause significant pharmacokinetic interactions with 2.5 mg of the drug [51]. Interestingly, grapefruit segments and grapefruit extract seem to influence the pharmacokinetics of felodipine to a similar extent as the juice itself [52]. Therefore, unprocessed grapefruit should also be avoided when taking drugs known to interact with GFJ.

2.1.3.2 *Transporter studies*

The influence of grapefruit juice over drug transporters has only been recently recognized. Dresser *et al.* [12] reported that in a randomized five-way crossover clinical study with ten healthy volunteers, grapefruit juice and apple juice at 5% of normal strength did not alter P-glycoprotein mediated digoxin or vinblastine transport, while 6′7′-dihydroxybergamottin had modest inhibitory activity. OATP-mediated fexofenadine uptake, however, was inhibited by grapefruit, orange and apple juices at 5% normal strength. From these results, it was concluded that inhibition of intestinal transport plays a major role in the way GFJ influences drug disposition [13]. It is still not clear, however, what the mechanism of OATP inhibition is, as analysis of human duodenal biopsy samples showed that grapefruit juice did not alter the expression of OATP1A2 or MDR1 [16]. Therefore, more studies are needed to establish which transporters are most affected by grapefruit juice.

2.2 Orange juice

Most studies found that orange juice did not inhibit CYP3A [2,3], and is therefore frequently used as a negative control in clinical studies. Orange juice only contains trace amounts of furanacoumarins, which are the compounds responsible for the inhibition of CYP3A. Interestingly, orange juice was shown to inhibit both P-glycoprotein and MRP2 [37,38] in rat intestine and Caco-2 cells respectively. In addition, orange juice was reported to reduce the AUCs of fexofenadine [12], atenolol [53], and celiprolol [54], by 30, 40, and 83%, respectively. This effect is speculated to be due to the inhibition of the OATP influx transporter by orange juice.

Seville (sour) orange juice, used primarily for the production of confectionery products [3], was shown to increase the AUC of felodipine [55] to a similar extent as GFJ. This effect is attributed to Seville orange juice's high bergamottin and DHB content [56]. Another study by Di Marco *et al.* [57] reported that GFJ and Seville orange juice both increased the bioavailability of dextromethorphan. The results of this study are difficult to interpret, however, since the bioavailability of the drugs was inferred from urine, rather than plasma, concentrations. Seville orange juice does not appear to inhibit P-glycoprotein, since it did not affect the pharmacokinetics of cyclosporine despite lowered CYP3A4 content [56]. To our knowledge, there are no studies regarding the effect of Seville orange juice on OATP.

2.3 Other citrus juices

Pomelo juice, another citrus juice similar to grapefruit juice, has also been investigated for its effect on drug disposition. As a relative of grapefruit, some varieties of pomelo also contain furanocoumarins which can inhibit CYP3A and P-glycoprotein. Both fruits seem to have similar quantities of 6′7′-dihydroxybergamottin, but pomelo has significantly less bergamottin than grapefruit [58,59]. In human liver microsomes, pomelo juice inhibited 6β-hydroxylation of testosterone by 37 – 76%, depending on the subspecies of the fruit [60]. In Caco-2 cells, grapefruit juice and pomelo juice inhibited the P-glycoprotein-mediated transport of digoxin [61] as well as Rhodamine-123 [62]. Pomelo juice also appeared to increase the AUC of cyclosporine by only 20% in a clinical trial with 12 healthy volunteers [58]. It is not clear whether this effect is clinically significant.

Lime and lemon juices have also been studied *in vitro* and in clinical trials. In contrast to grapefruit and pomelo juices, lime and lemon juices did not inhibit P-glycoprotein-mediated digoxin [61] or Rhodamine 123 [62] transport in Caco-2 cells. Instead, lemon and lime juices seemed to enhance digoxin transport [61].This latter phenomenon is thought to be due to the cytotoxicity of these juices, since the viability of Caco-2 cells decreased noticeably at concentrations above 5% juice, possibly leading to an increase in cell membrane permeability [61]. In contrast, grapefruit and pomelo juices enhanced Caco-2 cell viabilities at concentrations higher than 30% [62], a phenomenon which is not completely understood.

Lime juice inhibited CYP3A-mediated hydroxylation of 6β-testosterone in human liver microsomes in a mechanism consistent with modest irreversible inhibition [63]. In a clinical trial with eight volunteers, lime juice did not alter the mean values for the pharmacokinetics of felodipine, but it more than doubled the AUC and the peak plasma concentrations of felodipine in two subjects. These effects are believed to be due to the high levels of bergamottin in lime juice, since the fruit is not known to contain 6′7′-dihydroxybergamottin [63]. While lime juice does inhibit CYP3A *in vitro*, there is not sufficient evidence from clinical trials to establish it as a significant inhibitor of the enzyme in humans.

Tangerine juice contains high levels of a flavonoid named tangeretin, which has been shown to stimulate the activity

of CYP3A4 in human liver microsomes [64-66]. A recent study compared the effect of tangeretin on midazolam hydroxylation *in vitro*, and the influence of tangerine juice on midazolam metabolism in eight healthy volunteers [67]. Tangeretin increased the formation of 1′-hydroxymidazolam by over 200% in human liver microsomes, suggesting enzyme activation *in vitro*. In a clinical study, the total AUC (0 – 11 h) was not significantly affected by tangerine juice. These findings suggest that tangerine juice is probably not a significant inducer of CYP3A4 in humans.

The effect of citrus soft drinks on CYP3A activity was examined following a case report which suggested a possible interaction between the carbonated soft drink Sun Drop® and cyclosporine [68]. Sun Drop® and Fresca® were co-administered with cyclosporine to 12 healthy volunteers in a randomized crossover study [69]. Both of these drinks were confirmed to contain bergamottin. Fresca® is believed to contain 83 times as much bergamottin as Sun Drop®. Neither drink was found to significantly affect cyclosporine pharmacokinetics, while grapefruit juice, the positive control, increased the drug's AUC by 186%.

2.4 Apple juice

To our knowledge, there have been no *in vitro* or clinical studies investigating the effect of apple juice on drug metabolism. There is evidence, however, that apple juice inhibits the OATP transporter. *In vitro* studies in transfected HeLa cells showed that 5% apple juice nearly eliminated rat oatp1 and oatp3 activities. Furthermore, in a clinical study with ten healthy volunteers, administration of apple juice reduced the AUC of fexofenadine to < 30% of controls, suggesting significant inhibition of the OATP transporter in humans. In the same study, the reduction in the AUC was similar with orange juice, while grapefruit juice reduced the AUC of fexofenadine to approximately 40% of controls.

2.5 Cranberry juice

Cranberry juice has become a popular prophylactic treatment for the prevention of urinary tract infections [70-72]. This health benefit is believed to be due to proanthocyanidins found naturally in cranberries, which prevent bacteria from adhering to uroepithelial cells [73,74]. Cranberries are also a rich source of phytochemicals, such as flavonol glycosides, anthocyanins, proanthocyanidins, and organic and phenolic acids [75]. These chemicals have the potential to decrease oxidative stress and inflammation, and to induce apoptosis in cancer cells [76]. The antioxidant activities of some of its flavonol glycosides even exceed that of Vitamin E [77]. Furthermore, cranberries have been shown to reduce the severity of atherosclerosis and ischemic stroke [76]. Hence, cranberries are believed to confer protective effects against cardiovascular disease and certain types of cancer such as breast, colon, prostate and lung [78].

Based on anecdotal case reports, cranberry juice was rumored to interact with warfarin through inhibition of CYP2C9 [79,80]. A clinical trial with 14 volunteers examined the effect of cranberry juice on the metabolism of flurbiprofen, a probe for CYP2C9 [81]. This study showed that cranberry juice did not inhibit CYP2C9 activity *in vitro*, nor did it influence CYP2C9 activity in humans. Cranberry juice also did not affect the prothrombin time of warfarin in seven volunteers [82]. Another pharmacokinetic study examined the impact of cranberry juice on warfarin, tizanidine, and midazolam, probes for CYP2C9, CYP1A2, and CYP3A4, respectively, in ten healthy volunteers [74]. Cranberry juice had no significant effect on the pharmacokinetics of the drugs, except for a slight decrease (7%) in the AUC of warfarin. The anticoagulant effect of the drug was not changed. Cranberry juice was also reported to have no significant effect on the pharmacokinetics of cyclosporine in a clinical trial with 12 volunteers [58]. Based on these studies, it is unlikely that cranberry juice influences CYP2C9 or CYP3A4 in humans.

2.6 Pomegranate juice

The popularity of pomegranate juice has increased significantly in the United States in the last decade, after clinical studies suggested that its consumption could decrease the risk of cardiovascular disease, suppress prostate and breast cancers, and possibly augment the effect of HIV treatment regimens [8,83-87]. The numerous health benefits of pomegranate juice are attributed to the high polyphenol content of the fruit. Many of these polyphenols, such as quercetin, kaempferol and gallic acid, have also been shown to inhibit CYP3A activity [88-92]. Ellagic acid, one of the most powerful antioxidants in pomegranate juice, has been reported to inhibit CYP2A2, 3A1, 2C11, 2B1, 2B2 and 2C6 in rat liver microsomes [93]. Caffeic acid, a phenolic acid in pomegranate juice was reported to slightly increase rat UDP-glucuronosyltransferase *in vivo*, but had no effect on the other metabolic enzymes [94]. Whole pomegranate juice (5% v/v) was found to inhibit 97% of CYP3A in human liver microsomes [10]. The IC_{50} for the inhibition of CYP3A, as measured by the hydroxylation of triazolam, is estimated to be between 0.5 and 1% [95].

In vivo studies in rats have shown that pomegranate juice impairs the activity of CYP3A, but only in the intestine [96]. The observed inhibition was quantitatively similar to that with grapefruit juice. Compared to controls, both pomegranate juice and grapefruit juice increased the AUC and the peak plasma concentration of carbamezapine by almost 50%. The half-life of the drug was not affected, suggesting that hepatic metabolism was not inhibited by either juice.

Clinical trials suggest that pomegranate juice does not inhibit the activity of CYP3A in humans. A randomized crossover clinical trial showed that the AUC and peak plasma concentration of midazolam were not altered by the consumption of 8 oz of pomegranate juice in 13 healthy volunteers [95]. Grapefruit juice, however, led to an increase of 65% in AUC compared to water. Another study examined

the effect of pomegranate juice on simvastatin, another CYP3A substrate [97]. In spite of the high dose of pomegranate juice (900 ml, or approximately 30 oz, for 3 days), no interaction was observed. Based on these clinical trials, it is unlikely that pomegranate juice would influence the metabolism of CYP3A substrates in humans.

A recent study has shown that pomegranate juice can inhibit the activity of CYP2C9 in rats [98]. While there have been no trials in humans, this study raises a concern, since CYP2C9 metabolizes 15% of prescription drugs, including the anticoagulant, warfarin. Warfarin has a narrow therapeutic index, meaning that a slight variation in its metabolism could lead to significant side effects, such as internal bleeding and gangrene of the skin. Furthermore, patients taking warfarin might also drink pomegranate juice for its cardiovascular protective properties. Therefore, the effect of pomegranate juice on CYP2C9 would need to be examined before administering it to patients taking drugs which are substrates of this enzyme.

2.7 Red wine and grape juice

Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenolic compound found in high concentration in red wine and grape juice [99]. Resveratrol exists in the *cis-* and *trans-* forms, but it is the *trans-* form which is responsible for its health benefits [100], and it is the form that will be discussed in this article. It is believed to be a powerful cancer chemotherapeutic agent, and has been reported to prevent cellular events leading to tumor initiation, promotion and progression [99]. Furthermore, resveratrol has also been shown to be protective against coronary heart disease by preventing platelet aggregation and regulating eicosanoid synthesis in human platelets and neutrophils [101]. Drug metabolism studies, however, have suggested that resveratrol also inhibits several cytochrome P450 enzymes, which could be dangerous for individuals who are taking certain prescription drugs.

In human recombinant enzyme systems, resveratrol inhibited CYP1A1 and CYP1B1, and it also inactivated CYP1A2 in mechanism-based fashion [99]. In human liver microsomes, resveratrol inhibited CYP1A1 but not CYP1A2 [101]. In another human microsome study, resveratrol and red wine solids (RWS), prepared by evaporating red wine to dryness and reconstituting it with 20% natural-strength buffer, both appeared to irreversibly inhibit CYP3A4 and non-competitively inhibit CYP2E1 [102]. Therefore, the inhibitory properties of red wine could also be due to RWS, some of which might also exhibit chemopreventive properties.

Whole grape juice was also examined in human liver microsomes, and its IC_{50} for CYP3A-catalyzed midazolam hydroxylation was 4.4 and 5.9% with and without pre-incubation, respectively. These values place the inhibitory potency of grape juice between pomegranate juice (IC_{50}s of 11.3 and 34.2% with and without preincubation, respectively) and grapefruit juice (IC_{50}s of 0.09 and 0.32% with and without preincubation, respectively) [11].

The interaction between grape juice and flurbiprofen, a substrate for CYP2C9, was examined in a clinical trial with 14 volunteers. The AUC and the maximum plasma concentration of flurbiprofen were not affected by grape juice, suggesting that grape juice is not a significant inhibitor of CYP2C9 [81].

A clinical study with 12 healthy men examined the pharmacokinetics of cisapride when it was administered with water, grapefruit juice, and red wine [103]. Grapefruit juice increased the AUC and the maximum plasma concentration of cisapride by 51 and 68%, respectively, compared to water. Red wine increased these values by 15 and 7%, respectively, which were not statistically significant.

In another clinical study, red wine caused 'dose dumping' of felodipine in four out of eight subjects. This phenomenon, occasionally observed with extended-release tablets, is characterized by delayed absorption and a rapid increase in plasma concentration of the drug [63]. In this particular study, the surge in plasma concentration of the drug could be due to rapid dissolution of the tablet coating by ethanol. One subject experienced adverse reactions, characterized by palpitations and facial flushing. Overall, red wine actually slightly decreased the AUC of felodipine, although this change was not statistically significant, suggesting that red wine does not significantly inhibit the activity of CYP3A4 [63].

3. Discussion: possible reasons for discrepancies between *in vitro* and clinical studies

In vitro studies have demonstrated that several different types of fruit juice have the capacity to influence drug disposition, but many of these interactions are not clinically significant (see **Table 2**). There are several reasons that could explain this lack of association: i) the concentration of the inhibitors might not be high enough in the juice; ii) the inhibitors might be metabolized or transported out of the target cells; iii) the concentration of inhibitors might be higher in the intestine than in the liver; iv) there could be differences in the site of action of the inhibitor between the intestines and the liver; v) there could be alternate drug metabolism pathways in the body; and vi) there might be species differences in drug-metabolizing enzymes, in the cases where the juices were shown to interact with drugs in animals.

Grapefruit juice is the only fruit juice which has been shown to interact with over 30 prescription drugs. As an irreversible inhibitor of the enzyme, grapefruit juice permanently inactivates CYP3A, forcing the body to regenerate the enzyme *de novo*. The regeneration half-life of the enzyme is approximately 23 h, therefore drugs taken many hours after the juice are still affected [19].

Grapefruit juice, as well as bergamottin, DHB, and furanocoumarin dimers, also inhibit other major cytochrome

Table 2. Interaction of fruit juices with drug metabolism enzymes *in vitro* and in humans.

Juice	*In vitro* interactions	Clinical interactions
Grapefruit	Inhibition of CYPs 1A2, 2C9, 2C19, 3A and 2D6 in human liver microsomes [36] Inhibition of PgP and MRP2 in Caco-2 cells [38] Activation of PgP in MDCK-cells [39] Inhibition of OATP transporter in HeLa cells [12]	Inhibition of orally administered drugs, which are CYP3A substrates and have high presystemic extraction, as reviewed in [6,14,15,20,109] Inhibition of OATP transporter Probe: fexofenadine [12]
Orange	Inhibition of Pgp and MRP2 in rat intestines [37] and Caco-2 cells [38]	Inhibition of OATP transporter Probes: fexofenadine [12], celiprolol [54]
Seville orange	N/A	Inhibition of CYP3A Probe: felodipine [55]
Pomelo	Inhibition of CYP3A in human liver microsomes [60] Inhibition of Pgp in Caco-2 cells [61,62]	Inhibition of CYP3A Probe: cyclosporine [58]
Lime	Inhibition of CYP3A in human liver microsomes [63]	Inhibition of CYP3A in only 2 out of 8 volunteers Probe: felodipine [63]
Tangerine	Induction of CYP3A in human liver microsomes [67]	No inhibition of CYP3A Probe: midazolam [67]
Cranberry	No inhibition of CYP2C9 [81]	No inhibition of CYP2C9 Probes: flurbiprofen [81], warfarin [74] No inhibition of CYP1A2 Probe: tizanidine [74] No inhibition of CYP3A4 Probes: midazolam [74], cyclosporine [58]
Pomegranate	Inhibition of CYP3A in human liver microsomes [10]	No inhibition of CYP3A Probe: midazolam [95]
Grape	Inhibition of CYP3A in human liver microsomes [11] Inhibition of CYP2C9 in human liver microsomes [81]	No inhibition of CYP2C9 Probes: flurbiprofen [81], warfarin [74]
Red wine	N/A	No inhibition of CYP3A4 Probes: cisapride [103], felodipine [63]

P450s *in vitro*, such as CYP 1A2, 2C9 and 2D6, *in vitro*, with IC_{50}s between 100 and 5000 nM [36]. To our knowledge, these interactions have not been shown to be clinically significant. The IC_{50}s in the CYP3A4-mediated drug oxidations for the furanocoumarin dimers ranged from 3 to 15 nM, and for bergamottin and DHB these values were between 250 and 1700 nM. Therefore, on average, the CYP3A4-mediated reactions had lower IC_{50}s, although there was some overlap between the values. Another possible explanation for the lack of clinical significance could be the existence of other pathways in the body which metabolize substrates of these enzymes. Also, clinically significant quantities of CYPs other than CYP3A are not present in the gastrointestinal enteric mucosa.

Orange juice does not inhibit CYP3A, and this is believed to be due to its low concentration of furanocoumarins. Orange juice does reduce the bioavailability of fexofenadine and celiprolol, and this effect is thought to be due to the inhibition of the OATP influx transporter, an interaction which was also observed *in vitro* [12]. Orange juice was also found to inhibit the P-glycoprotein transporter *in vitro* [37,38], and this effect is attributed to polymethoxylated flavones in the juice [104]. To our knowledge, there have not been studies to determine whether this inhibition is clinically significant.

Seville orange juice has been shown to inhibit the metabolism of felodipine [55] to the same extent as grapefruit juice. The inhibitory effects of Seville orange juice are probably due to the high concentration of bergamottin and DHB found in the juice. Furthermore, Seville orange juice has been reported to decrease the concentration of CYP3A4 of enterocytes, suggesting mechanism-based inhibition [3,56]. Seville orange juice is also unlikely to inhibit P-glycoprotein, since it was reported not to interact with cyclosporine despite lowered CYP3A4 content [56].

For most other citrus juices, there have not been enough studies to evaluate their inhibitory capacities and the reasons for the lack of correlation between *in vitro* and *in vivo* data. Lime juice inhibited CYP3A in human liver microsomes, but the results of the clinical study were not clear. Overall, the AUC of felodipine doubled in only two out of eight subjects, an effect that could be due to chance [63]. Citrus soft drinks such as Sun Drop® and Fresca® were also reported to contain very high quantities of bergamottin, yet no interaction was observed with cyclosporine [69]. Bergamottin is a significantly weaker inhibitor of CYP3A

than DHB, therefore the lack of interaction with the citrus soft drinks is not surprising.

Based on clinical data, it appears that only fruit juices with high DHB content can lead to clinically significant interactions with CYP3A4. This could be the reason that grapefruit juice and Seville orange juice, which contain very high quantities of DHB, inhibit CYP3A4 in humans. Pomelos, which contain as much DHB as grapefruits, inhibited CYP3A activity *in vitro* [60] and also in one clinical study with cyclosporine [58]. The increase in cyclosporin's AUC was only 20%, however, therefore the clinical relevance of this interaction might not be significant. Tangerine juice was believed to stimulate CYP3A activity rather than inhibit it, due to the presence of a flavonoid named tangeretin [64-66,105]. In human liver microsomes, tangeretin increased the formation of 1′-hydroxymidazolam by 200%, suggesting significant activation of CYP3A; but in a clinical trial, tangerine juice did not affect the overall AUC. Based on this study, tangerine juice does not appear to induce CYP3A in humans.

Cranberry juice was believed to inhibit CYP2C9, based on anecdotal case reports. In human liver microsomes and in clinical studies, no interaction was observed between cranberry juice and flurbiprofen [81]. Cranberry juice also did not interact with cyclosporine in humans [58], suggesting no effect on CYP3A4 metabolism. The case of cranberry juice shows the importance of conducting controlled pharmacokinetic trials before drawing conclusions from anecdotal case reports. It is possible that the adverse drug interactions reported in case studies are due to chance association and not a result of food–drug interactions.

Based on *in vitro* and animal studies, pomegranate juice appeared to be as strong an inhibitor of CYP3A4 as grapefruit juice [10]. In the animal studies, pomegranate juice also appeared to have a similar mechanism of action as grapefruit juice, since it only inhibited enteric CYP3A4 [96]. The *in vitro* studies, however, showed that pomegranate juice was a reversible inhibitor of the enzyme, in contrast to grapefruit which irreversibly inactivated it [95]. A clinical study later showed that pomegranate juice did not influence the pharmacokinetics of the CYP3A4 probe, midazolam [95]. The lack of correlation between *in vitro* and clinical studies is believed to be due to pomegranate juice being a reversible inhibitor of the enzyme. It is still interesting to note, however, that pomegranate juice was a potent inhibitor of CYP3A in rats. This discrepancy could be due to species differences in the isoforms of CYP3A. In humans, the metabolism of midazolam is carried out by CYP3A4 and CYP3A5, whereas in rats the responsible enzymes are CYP3A1, CYP3A2 and also members of the CYP2C family [95,106,107].

Grape juice and red wine were suspected to interact with some cytochrome P450 enzymes, due to their high concentration of resveratrol. In human liver microsomes, resveratrol inhibited CYP1A1 [101] and whole grape juice inhibited CYP3A [11]. Clinical studies, however, reported that grape juice did not inhibit CYP2C9 [81], and red wine did not significantly interact with CYP3A4 [63,103]. It is important to note, however, that in one of the trials red wine caused dose-dumping in four out of eight volunteers. It is possible that the delayed, rapid increase in drug plasma concentration was due to felodipine being in an extended-release formulation tablet. While red wine was not tested *in vitro*, grape juice was found to be a reversible inhibitor of CYP3A in human liver microsomes [11], possibly explaining the lack of interaction in humans.

4. Conclusion

In summary, grapefruit juice has been shown to interact with the most number of compounds in clinical studies. This interaction is probably due to its high concentrations of DHB and/or the spiroester dimers, which are very potent irreversible inhibitors of enteric CYP3A. Orange juice, which does not contain significant quantities of DHB, does not interact with CYP3A. However, orange juice has been shown to inhibit OATP-mediated transport in clinical studies. Seville orange juice and pomelo juice, which contain as much DHB as grapefruit juice, have also been shown to interact with some drugs, but far less than GFJ. For the latter two juices, however, there have not been enough studies to evaluate their inhibitory potencies, as they are not consumed commonly by the public.

Based on clinical studies, it appears that the juices that inhibit CYP3A contain significant quantities of DHB. It is important to note, however, that inhibition of CYP3A can be achieved without DHB. In fact, some studies attribute the inhibition of CYP3A enzyme to the net effect of several different types of furanocoumarins and heterodimers [59,108]. The furanocoumarins dimers, in particular, have been shown to be very potent inhibitors of CYP3A [27]. In human microsomal studies, for example, a furanocoumarins dimer cocktail appeared to inhibit CYP3A as much as grapefruit juice, but omission of any of the compounds resulted in decreased inhibition [59]. Interestingly, these compounds produced both reversible and mechanism-based inhibition, suggesting that the irreversible inhibition might not be the only mechanism by which CYP3A activity is reduced. Given the complexity of the interactions between food products on prescription drugs, the inhibition of CYP3A cannot be predicted without controlled pharmacokinetic studies.

5. Expert opinion

The possibility of interactions between food products and prescription drugs is a concern for individuals on multiple drug regimens. The interaction between grapefruit juice and CYP3A4, which was discovered nearly two decades ago, raised a concern regarding the influence of fruit juices on drug-metabolizing enzymes. Based on the data that has

been collected, however, it appears that many of the interactions observed *in vitro* are not clinically significant. Grapefruit juice, for example, affects only orally administered drugs with a high presystemic extraction. Seville orange juice, a sour juice used primarily for confectionery products, has only been shown to inhibit the CYP3A-mediated metabolism of felodipine and possibly dextromethorphan. Interestingly, orange juice does not interact with CYP3A4, but it does inhibit the organic anion transport protein (OATP).

The inhibition of CYP3A by grapefruit juice and Seville orange juice is attributed to the presence of naturally occurring furanocoumarins. The most potent of these inhibitors is 6′7′-dihydroxybergamottin (DHB), but the inhibition appears to be due to the combined effect of all of the furanocoumarins in the juice. In fact, a recent clinical trial showed that extraction of these compounds from grapefruit juice completely removes its inhibitory potency. Therefore, it is theoretically possible to commercialize a furanocoumarin-free grapefruit juice, although its taste is altered by the extraction process.

In order to determine whether a fruit juice can influence drug-metabolizing enzymes in humans, one must carry out clinical studies rather than rely on *in vitro* experiments, anecdotal case reports, or animal studies. *In vitro* studies frequently show interactions that are not clinically significant, since not all metabolic pathways can be reproduced *in vitro*. Tangerine juice, for example, activates CYP3A4 in human liver microsomes, but there was no effect on this enzyme in a clinical study. Anecdotal case reports also need to be analyzed carefully because they are usually based on single individuals whose medical history could confound the results. Cranberry juice was reported to inhibit CYP2C9 in case reports, but such interaction could not be reproduced in clinical trials. Neither do animal studies substitute for human studies, as there are significant species–species differences between drug-metabolizing enzymes. A good example of this lack of correlation is pomegranate juice, which inhibited CYP3A4 in rats but not in humans.

Studies with fruit juices also need to take into consideration the dose of juice administered to volunteers. Grapefruit juice, for example, affects intestinal CYP3A only when consumed in normal (10-oz) doses. Some studies have found an effect on the hepatic enzyme when multiple servings doses of double- or triple-strength grapefruit juice were consumed by volunteers. Since such extreme doses are not commonly consumed by the public, the methodology of each study needs to be carefully examined before making conclusions regarding the effect of fruit juices on various aspects of drug disposition.

Fruit juices have also been evaluated regarding their effects on drug transporters. OATP appears to be affected by grapefruit juice, orange juice, and apple juice at 5% normal strength. The inhibition of a drug transporter can significantly impact the disposition of certain drugs. In the case of the OATP influx transporter, the systemic exposure to its substrates decreased by 30 – 80% when co-administered with orange juice. Interestingly, P-glycoprotein does not seem to be affected by fruit juices in clinical trials, although grapefruit and orange juice did inhibit this transporter in some *in vitro* studies.

Based on current reports from clinical trials, it appears that most *in vitro* interactions between juices and prescription drugs are not clinically significant, but there are exceptions. Grapefruit juice and Seville orange juice inhibit CYP3A in humans, probably due to the presence of DHB. Furthermore, grapefruit juice, orange juice, and apple juice also inhibited the OATP transporter in clinical studies. It is possible that other fruit juices also influence drug-metabolizing enzymes and transporters in humans, but more studies need to be carried out before specific conclusions can be made.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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