Influence of Dietary Substances on Intestinal Drug Metabolism and Transport

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Abstract:

Successful delivery of promising new chemical entities *via* the oral route is rife with challenges, some of which cannot be explained or foreseen during drug development. Further complicating an already multifaceted problem is the obvious, yet often overlooked, effect of dietary substances on drug disposition and response. Some dietary substances, particularly fruit juices, have been shown to inhibit biochemical processes in the intestine, leading to altered pharmacokinetic (PK), and potentially pharmacodynamic (PD), outcomes. Inhibition of intestinal CYP3A-mediated metabolism is the major mechanism by which fruit juices, including grapefruit juice, enhances systemic exposure to new and already marketed drugs. Inhibition of intestinal non-CYP3A enzymes and apically-located transport proteins represent recently identified mechanisms that can alter PK and PD. Several fruit juices have been shown to inhibit these processes *in vitro*, but some interactions have not translated to the clinic. The lack of *in vitro-in vivo* concordance is due largely to a lack of rigorous methods to elucidate causative ingredients prior to clinical testing. Identification of specific components and underlying mechanisms is challenging, as dietary substances frequently contain multiple, often unknown, bioactive ingredients that vary in composition and bioactivity. A translational research approach, combining expertise from clinical pharmacologists and natural products chemists, is needed to develop robust models describing PK/PD relationships between a given dietary substance and drug of interest. Validation of these models through well-designed clinical trials would facilitate development of common practice guidelines for managing drug-dietary substance interactions appropriately.

Article:

INTRODUCTION

Interpatient differences in response to therapeutic agents represent one of the most challenging complications in clinical practice. Such complications can delay, even prevent, optimal treatment outcomes, which can negatively impact quality of life and health care costs. Genetic, pathophysiologic, and environmental factors all contribute to variation in drug response, which is in part due to large interindividual differences in processing xenobiotics *via* absorption, distribution, and elimination. Significant resources continue to be invested in delineating genetic factors associated with variation in drug disposition, and in turn drug response, with the promise of “personalized medicine” [1-3]. Comparatively less attention has been directed toward non-genetic factors, which are equally important in determining drug response [4], and whose contribution increases with age [5]. Because ingestion of dietary substances, as foods or supplements, undoubtedly constitutes the largest portion of environmental exposure to

xenobiotics, evaluation of the influence of dietary substances on drug disposition is prudent to improving the understanding of interindividual differences in response to therapeutic agents.

Dietary substances perhaps have the greatest impact on drug disposition processes in the intestine, as most drugs and dietary substances enter the body by the oral route and are absorbed subsequently by enterocytes. Like hepatocytes, enterocytes express myriad metabolizing enzymes and transport proteins that influence, at least in part, the extent of systemic drug exposure [6, 7]. The clinical significance of the intestine as a contributor to drug disposition and as a site for drug-drug interactions (DDIs) is widely recognized. Incorporation of intestinal biochemical processes in DDI prediction models is the topic of several recent reviews and original research articles [8-15].

Although dietary substances are regulated as food, bioactive compounds in these substances can act like drugs. Presumed bioactive compounds often are extracted and sold as dietary or herbal supplements. The ever-increasing popularity of certain foods and dietary supplements as a means to decrease health care costs *via* self-diagnosis and treatment is due in part to the widely held view that these products are safer, "natural" alternatives to prescription, as well as non-prescription, drugs [16, 17]. Evaluation of drug interaction liability of new drug candidates is strictly defined [18, 19], whereas that for foods and supplements is not. Consequently, robust guidelines on the evaluation of potential drug-dietary substance interactions are essentially non-existent. Lack of guidance in this area has led to a multitude of studies that often are difficult to compare, inconclusive, and fail to meet strict definitions required to make informed clinical and regulatory decisions. The current review focuses on new findings and developments over the last two years in drug-dietary substance interaction research and addresses concerns regarding interpretation of associated studies.

OVERVIEW OF DRUG-DIETARY SUBSTANCE INTERACTIONS

A drug-dietary substance interaction is defined as the result of a physical, chemical, physiologic, or pathophysiologic relationship between a drug and a nutrient(s) present in a food, nutritional supplement, or food in general [20]. Such an interaction manifests clinically as compromised nutritional status due to addition of a drug or altered pharmacokinetics (PK) and/or pharmacodynamics (PD) of a drug or dietary substance. Like drugs, dietary substances can act as objects or precipitants [21], the latter of which can increase systemic drug exposure, augmenting the risk of adverse events and toxicity, or decrease systemic drug exposure, leading to therapeutic failure. These interactions are challenging to assess because, unlike most drug products, dietary substances are mixtures, composed of multiple, and usually unknown, bioactive ingredients. A mechanistic understanding of the varied effects of dietary substances on drug disposition would form a basis for optimizing pharmacotherapy by minimizing potential unwanted effects.

Clinical Considerations

Dietary habits often are an overlooked topic of discussion during clinician visits, as well as during clinical trial design. The general lack of awareness of clinicians to identify and properly manage drug-dietary substance interactions may predispose patients to unfavorable outcomes. The risk of experiencing a significant event depends on several factors. While a drug-dietary substance interaction may occur in any patient, those with weakened physiologic function, such

as the elderly, immunocompromised, and critically ill, are at the highest risk of experiencing untoward effects [22]. Management of these relatively unexplored interactions is a challenge in clinical practice. The clinician must identify short- and long-term consequences, determine the need for dosing and/or timing adjustments for the drug(s), and consider alternative treatment approaches [23]. Understanding underlying mechanisms of the interaction and causative bioactive compounds will facilitate making the most appropriate decision. However, prospective and systematic investigations on mechanisms and outcomes of many interactions are insufficient or lacking altogether. Clinical interaction studies often do not support *in vitro* observations [24]. These *in vitro-in vivo* discordances raise questions about how research is conducted and interpreted. Taken together, practical approaches in the management of these interactions are difficult to formulate. Development of common practice guidelines to provide a consistent and comprehensive recommendation on avoiding or assessing drug-dietary substance interactions can be achieved only by designing and conducting robust clinical studies.

Dietary Substances as Precipitants of Altered Drug Exposure and Response

Dietary substances as precipitants can alter drug absorption, distribution, and/or elimination *via* physicochemical and biochemical mechanisms. Physicochemical mechanisms include inactivation of the drug by the dietary substance. For example, enteral feeding formulas are physically incompatible with certain medications. The antiepileptic agent, phenytoin, can bind to proteins and salts in enteral formulations, resulting in reduced phenytoin absorption, reduced serum concentrations, and potentially, inadequate seizure control [25]. Some tetracyclines and fluoroquinolones can bind to divalent cations in dairy products (*e.g.*, calcium), resulting in reduced drug absorption and potential therapeutic failure [26, 27]. Biochemical mechanisms include alterations of gastroenterologic processes, interference with co-factor formation or function, and modification of drug metabolizing enzyme/transporter function by the dietary substance. For example, high fat meals can increase drug absorption by improving solubility or stimulating gastrointestinal enzymes and bile flow [28]. The antifungal agent, griseofulvin, and antiviral agent, saquinavir, are recommended to be taken with such meals [29]. Food and beverages in general can delay gastric emptying or change gastric pH, causing reduced absorption of some drugs, including penicillins and proton pump inhibitors [30]. Vitamin K-rich foods, such as dark green leafy vegetables, are examples of dietary substances that interfere with co-factor function [31]. These foods should be consumed cautiously with the anticoagulant, warfarin, as they can interfere with vitamin K metabolism and increase risk of bleeding or clot formation [32]. Fruit juices are examples of dietary substances that modify drug metabolism/transport and are the focus of this review. Other examples of drug-dietary substance interactions are discussed comprehensively in several sources [33-36].

MECHANISMS OF ALTERED SYSTEMIC DRUG EXPOSURE *VIA* INHIBITION OF INTESTINAL BIOCHEMICAL PROCESSES

The gastrointestinal tract is exposed continuously to a variety of xenobiotics, the majority of which are components of the diet. Fruit juices are touted frequently as healthy foods due to high antioxidant content, which is believed to slow onset of disease and aging [37]. These ubiquitous products are ready-made, easily obtained, and affordable. They have become highly recommended supplements to routinely prescribed and over-the-counter drugs and/or monotherapy for prevention, treatment, and maintenance of common diseases (*e.g.*,

hypercholesterolemia, hypertension, and diabetes mellitus). The prevalence of these chronic conditions, and associated use of medications and fruit juices, is expected to rise [38-40].

Compared to drugs, less attention has been given to the possibility that dietary substances can influence drug disposition *via* modulation of drug metabolizing enzymes and transporters. Fruit juices have been shown to inhibit metabolism and active apical efflux/uptake processes in the intestine [41]. Inhibition of metabolism and active efflux would be expected to increase, whereas inhibition of active uptake would be expected to decrease, systemic drug exposure. These biochemical mechanisms of the intestine are highlighted in the current review.

Cytochrome P450 3A

Cytochrome P450 (CYP) enzymes constitute the major catalysts of phase I drug biotransformation [42]. The CYP3A subfamily, consisting primarily of CYP3A4 and CYP3A5, is the most abundantly expressed in the intestine, representing, on average, approximately 80% of total immunoquantified CYP protein [43]. CYP3A is believed to be involved in the oxidative metabolism of over 50% of pharmaceutical agents [44]. Some fruit juices have been shown to inhibit enteric CYP3A, leading to clinical consequences [45]. Although several *in vitro* observations have translated to the clinic, generalizations about the effect of fruit juices on the metabolism of CYP3A substrates should be avoided since the effect may be substrate-dependent.

Grapefruit Juice. The grapefruit (*Citrus* × *paradisi*), particularly as juice, is one of the most extensively studied dietary substances shown to interact with an array of medications [46]. Grapefruit juice (GFJ) can enhance systemic drug exposure, by up to 1400%, by inhibiting CYP3A-mediated pre-systemic (first-pass) metabolism in the intestine [47]. Inhibition is localized largely in the gut, as reflected by a lack of effect on the PK of an intravenously administered substrate and on the elimination half-life of an orally administered substrate [48]. The increase in systemic drug exposure can be sufficiently large to produce untoward effects, including muscle pain with some HMG CoA reductase inhibitors (statins) and severe hypotension with some calcium channel antagonists [49]. Accordingly, the package insert of more than 40 drugs, encompassing a range of therapeutic classes, carries a warning to avoid concomitant GFJ intake.

The serendipitous observation of a PK/PD interaction between GFJ and the anti-hypertensive agent, felodipine [50], spurred numerous investigations of various drug-GFJ interactions. Modes of intestinal CYP3A inhibition by GFJ include reversible and mechanism-based [51], as well as destruction of the protein [52]. A number of causal ingredients were examined over a span of 15 years before a class of compounds, furanocoumarins, was established as a major mediator of the ‘GFJ effect’ in human subjects [53]. The discovery of the GFJ effect and subsequent investigations underscores the importance of the intestine as an organ of drug elimination and the possible importance of other dietary substances as modifiers of drug performance. Figure 1 highlights other key observations since the discovery of the felodipine-GFJ interaction in 1989 [54]. Table 1 summarizes the design and major results of recent healthy volunteer and patient studies reporting modest to significant PK interactions with medications that are CYP3A substrates.

Figure 1: Timeline of key observations in drug-grapefruit juice (GFJ) interaction research.

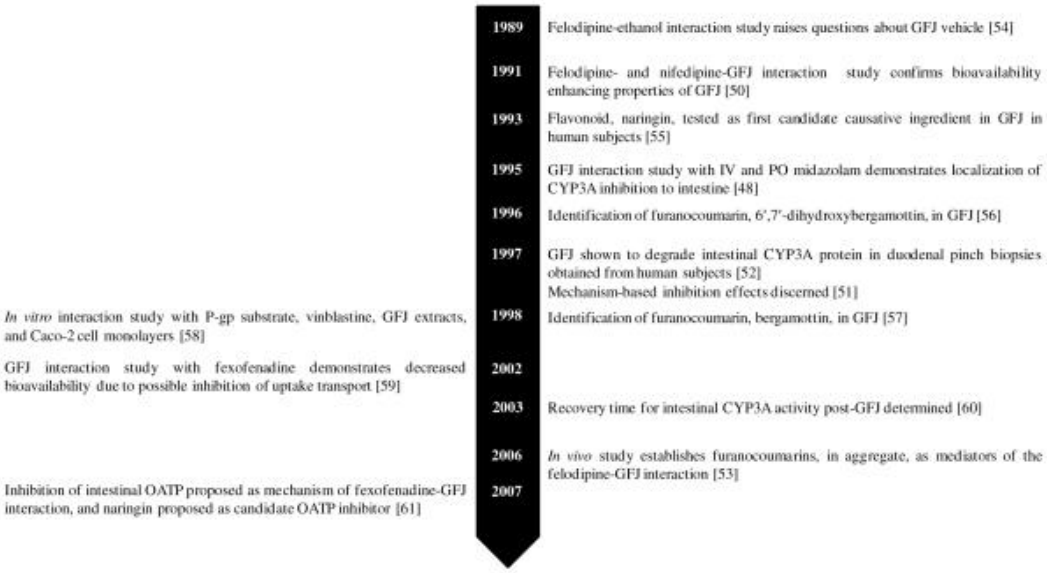

Table 1: Controlled clinical drug-grapefruit juice (GFJ) interaction studies reported since 2008

Subjects (n)	Drug Tested and Dosage	GFJ Product (Manufacturer) and Administration Regimen	Change in Mean AUC	Reference
Healthy volunteers (20)	Itraconazole 200 mg (20 mL oral solution) (day 3)	Concentrate: diluted to single strength (Kroger Brand; The Kroger Co., Cincinnati, Ohio) 240 mL tid × 2d 200 mL × 2 (day 3)	↑ 30% (women) (p = 0.01) ↑ 11% (men) (p = 0.27)	[62]
Healthy volunteers (18)	Cyclosporine 5 mg/kg × 1	Concentrate: diluted to single strength (NSP)	↑ 38%§ (p < 0.01)	[63]
		FC-free: single strength* 240 mL × 1	↓ 1.8%§ (p > 0.70)	
Healthy volunteers (19)	Dextromethorphan 10 mg (dissolved in 50 mL water) × 1	Concentrate: diluted to 0.25×, 0.5×, 1×, 2× strength (Mainfrucht GmbH & Co. KG, Gochsheim, Germany) 200 mL × 1	↓ excretion of CYP3A-dependent metabolite (MOM) by 1× and 2× strength† (p < 0.05)	[64]
Healthy men (8)	Budesonide 3 mg (as ER) × 1 (day 4)	Freshly squeezed (AstraZeneca R&D, Lund, Sweden) 200 mL tid × 3d 200 mL × 1 (day 4)	↑ 129%	[65]
Healthy volunteers (8)	Sertraline 75 mg × 1 (day 6)	250 mL tid × 5d 250 mL × 1 (day 6)	↑ 51% (p = 0.002)	[66]
Liver transplant recipients (30, equally divided into groups A, B, and C)	Tacrolimus NSP	Concentrate: diluted to 0.125× strength (Guangdong Foshan Co., China) (group B)	↑ 22% (p < 0.01)	[67]
		Single strength (Tianjin Chengbao Fresh Grapefruit Juice Co., China) (group C) 250 mL bid × 1 week	↑ 110% (p < 0.001)	
Healthy men (21)	Nilotinib 400 mg × 1	Concentrate: diluted to 2× strength (Kroger Brand; The Kroger Co., Cincinnati, Ohio) 240 mL × 1	↑ 29% (p = 0.004)	[68]

Cancer patients (8)	Sunitinib 25/37.5/50[‡] mg qd (6-week treatment cycle: 4 weeks on, 2 weeks off)	NSP 200 mL tid × 3d (day 25, 26, 27)	↑ 11% ($p < 0.05$)	[69]
Healthy volunteers (12)	Oxycodone 10 mg × 1 (day 4)	Single strength (Greippi Täysmehu; Valio Ltd., Helsinki, Finland) 200 mL tid × 5d	↑ 67% ($p < 0.001$)	[70]
Healthy volunteers (11)	Aliskiren 150 mg × (day 3)	Normal strength (Valio Greippitäysmehu; Valio Ltd., Helsinki, Finland) 200 mL tid × 5d	↓ 81% ($p < 0.001$)	[71]

[*]Manufactured from concentrate
[§]Median AUC
[†]CYP3A- and CYP2D6-dependent metabolites measured in urine only
[‡]Dose depends on type of cancer
GFJ, grapefruit juice; AUC, area under the curve; FC, furanocoumarin; MOM, 3-methoxymorphinan; ER, extended release; tid, three times daily; d, days; NSP, not specified; bid, two times daily; qd, daily

Pomelo Juice. The pomelo (*Citrus maxima*), or pummelo, is a large citrus fruit native to Asia and is consumed typically as the fresh fruit. The grapefruit is believed to be an accidental hybrid of the pomelo and sweet orange (*Citrus sinensis*) [72]. Accordingly, it is reasonable to expect furanocoumarins are present in pomelos. Indeed, juice prepared from some species of pomelo has been reported to contain furanocoumarins in concentrations comparable to those in GFJ [73]. Clinical interactions with tacrolimus [74] and cyclosporine [75] *via* enteric CYP3A inhibition, albeit modest, have been reported. A clinical study of pomelo juice evaluated the extent of inhibition based on the species of pomelo [76]. Freshly prepared juices from two varieties of fruit ('Guanximiyou' and 'Changshanhuyou') were given, on separate occasions, with felodipine (10 mg) to 12 healthy volunteers. Each juice was measured for furanocoumarin content and tested for inhibition of CYP3A activity (testosterone 6β-hydroxylation) in human liver microsomes prior to clinical testing; at 2.5% juice (v/v), extents of inhibition were ~30% (Guanximiyou) and <5% (Changshanhuyou), relative to control. The more potent juice increased both mean area under the curve (AUC) and maximum concentration (C_{max}) of felodipine, by ~40% ($p<0.05$), whereas the less potent juice increased these values by ~15% (NS) relative to water. Heart rate also was measured to determine effects on felodipine PD. Neither juice altered mean heart rate significantly. Unlike the tacrolimus and cyclosporine studies, the felodipine study acknowledged and attempted to account for PK variability with respect to furanocoumarin composition in the juice (see **DISCORDANCE BETWEEN *IN VITRO* AND CLINICAL STUDIES**).

A clinical study of six healthy men showed a significant interaction between pomelo juice and sildenafil (50 mg), indicated for erectile dysfunction and pulmonary hypertension [77]. Since sildenafil undergoes extensive intestinal first-pass metabolism by CYP3A (oral bioavailability ~40%), and pomelos contain furanocoumarins, an increase in systemic sildenafil exposure (relative to water) was expected. However, the juice significantly decreased mean AUC and C_{max} of sildenafil. The authors speculated the mechanism was either a physicochemical interaction between sildenafil and components of the juice or inhibition of an intestinal uptake process (see **Uptake Transport Proteins**). Unlike the aforementioned study with felodipine, furanocoumarins were not measured in the juice.

Cranberry Juice. The cranberry (*Vaccinium macrocarpon*) has long been considered a health food, touted for beneficial effects on diverse ailments [78]. More than 150 individual compounds have been identified [79]. As a rich source of phytochemicals, cranberries have shown anti-

atherosclerotic and anti-proliferative properties, which may be protective in cardiovascular disease and certain cancers [80]. Cranberry juice (CBJ) continues to maintain popularity, largely as prophylaxis and treatment for urinary tract infections (UTIs) [81]. An *in vivo* study in rats given CBJ and the CYP3A/Cyp3a substrate, nifedipine, indicated that the juice inhibited enteric Cyp3a activity to an extent comparable to that by GFJ [82]. A subsequent clinical study involving 12 healthy volunteers given cyclosporine and a single 240-mL glass of CBJ indicated no interaction [83]. However, use of cyclosporine as a CYP3A probe was not ideal since cyclosporine also is a substrate for the efflux transporter, P-glycoprotein (P-gp) (see **Efflux Transport Proteins**), and whether or not CBJ modulates intestinal P-gp activity is not known.

Two clinical trials using midazolam as a CYP3A/non-P-gp probe showed conflicting results. The first study involved 10 healthy volunteers given a thrice daily regimen of a commercially available CBJ concentrate (diluted 1:4) for 10 days and a single dose of midazolam (0.5 mg on day 5) [84]. Relative to water, a change in midazolam PK was not detected. The second study involved 16 healthy volunteers given a single dose of midazolam (5 mg) and three 240-mL glasses of 'double strength' CBJ [85]. Prior to the clinical study, five brands of juice were tested *in vitro* to identify a product to test *in vivo*. The most potent brand selected increased geometric mean AUC of midazolam significantly, by ~30% relative to water, with no effect on elimination half-life. Another feature of the second study was that *in vitro* bioactivity-guided fractionation was utilized to isolate and identify candidate CYP3A inhibitors. The clinical test juice, a concentrate, was fractionated to generate hexane-, chloroform-, butanol-, and aqueous-soluble fractions. The hexane- and chloroform-soluble fractions (50 μg/mL) inhibited CYP3A activity (midazolam 1′-hydroxylation) in human intestinal microsomes by ~80 and 60%, respectively, suggesting the CYP3A inhibitors resided in these more lipophilic fractions. The juice was purified further until three triterpenes were isolated (maslinic acid, corosolic acid, ursolic acid) as candidate causative ingredients, with IC_{50} values ≤10 μM) [86]. The discrepancy between the two clinical studies may be explained by the difference in concentration of bioactive components. Only one brand was tested in the first study [84], and various components (anthocyanins, flavonols, hydroxycinnamic acids, hydroxybenzoic acids, and catechins) were measured. The most abundant was the flavonol, rutin, but CYP3A inhibition potency was not evaluated *in vitro*. Recognizing the substantial variability of bioactive components in natural products, the second study [85] began with *in vitro* testing to inform selection of the most appropriate brand for clinical testing and to generate candidate enteric CYP3A inhibitors for further investigation.

Although not intestinal CYP3A metabolism-based, the presumed warfarin-CBJ interaction *via* inhibition of hepatic CYP2C9 continues to be a topic of debate. Case reports persist despite randomized clinical trials in healthy volunteers and stably anticoagulated patients demonstrating no evidence of a PK/PD interaction. One exception is a three-arm, randomized crossover study involving 12 healthy men given a single dose of warfarin (25 mg) alone or with a commercially available garlic or cranberry product, the latter a capsule formulation of a cranberry juice concentrate [87]. Subjects were treated with cranberry (or garlic) daily for three weeks. Warfarin was given after the second week. Warfarin PK and PD were assessed; PD were measured by the International Normalized Ratio (INR), platelet aggregation, and clotting factor activity. Compared to warfarin alone, cranberry increased area under the INR-time curve significantly, by 30%, but had no effect on warfarin PK, platelet aggregation, and clotting factor activity. The

increased area under the INR-time curve could have reflected the higher-than-average warfarin dose and/or "megadose" of cranberry, which was equivalent to 57 g of cranberry fruit daily and was more than triple the UTI prophylaxis "dose" recommendation [88]. A review published in 2010 discussed studies to date (including the aforementioned sole finding) and concluded that moderate consumption of CBJ does not affect anticoagulation and that inclusion of precautionary warnings in warfarin product labeling should be re-examined [89]. Nevertheless, warfarin labeling continues to advise patients to avoid taking cranberry juice or cranberry products [90]. A more thorough understanding of the CBJ product in question is necessary to ascertain whether or not CBJ can enhance systemic exposure to clinically relevant CYP2C9, as well as CYP3A, substrates in humans.

Pomegranate Juice. The pomegranate (*Punica granatum*) and associated by-products is one of the most popular superfoods on the market. Like CBJ, pomegranate juice is a complex mixture of polyphenolic compounds with high antioxidant potency [91]. Human *in vitro* and rat *in vivo* studies suggested that pomegranate juice can inhibit enteric CYP3A/Cyp3a activity (carbamazepine expoxidation) [92]. However, a subsequent clinical study involving 13 healthy men given 240 mL of pomegranate juice and a single oral dose of midazolam (6 mg) suggested minimal interaction, despite inhibition of CYP3A activity (triazolam hydroxylation) in human liver microsomes [93]. Likewise, another study involving 12 healthy subjects given a single dose of simvastatin (40 mg) after treatment with of a different brand of pomegranate juice (300 mL three times daily for three days) reported no interaction [94]. Generalizations about the enteric CYP3A inhibition potential of pomegranate juice are cautioned, as minimal to no information was provided about the test juices and their composition, precluding between-study comparisons. In addition, neither clinical study provided a sample size justification.

Recent anecdotal reports have suggested an interaction between pomegranate juice and warfarin, as assessed by an increase in INR [95, 96]. Although hepatic CYP2C9/enteric Cyp2c inhibition by pomegranate juice has been demonstrated in human liver microsomes and rats with the probe substrates diclofenac and tolbutamide [97], respectively, no clinical trials have been reported. One case report involving rosuvastatin, which undergoes minimal metabolism, described rhabdomyolysis possibly due to an interaction with pomegranate juice [98]. This observation has yet to be investigated experimentally.

Esterase

Ester prodrugs are designed commonly to increase drug absorption [99]. Upon ester bond cleavage through hydrolysis or oxidation, active drug is released. Major esterases that hydrolyze prodrugs include carboxylesterase, acetylcholinesterase, butyrylcholinesterase, paraoxonase, and arylesterase [100]. Esterases are localized in multiple tissues, particularly blood, liver, and intestine [101]. Esterase inhibition could lead to increased stability of the ester in the lumen and enterocytes, resulting in higher absorption of the ester and higher exposure to active metabolite *via* rapid hydrolysis in plasma.

Grapefruit Juice. Enalapril is a prodrug that is metabolized primarily by carboxylesterase to enalaprilat, an angiotensin converting enzyme inhibitor [102]. Lovastatin, indicated for hypercholesterolemia, is a prodrug that is hydrolyzed to the active acid by carboxylesterase, as well as oxidized to several inactive metabolites by CYP3A/Cyp3a; hydrolysis is considered the

major metabolic pathway [103]. Lovastatin also has been suggested to be a weak substrate for P-gp [104]. Effects on the apical-to-basolateral (absorptive) permeability and/or metabolism of enalapril and lovastatin by GFJ (diluted 1:3 from frozen concentrate) were evaluated in a human intestine-derived cell line (Caco-2) and human intestinal and liver S9 fractions [105]. Relative to 0% (v/v) juice (buffer), the permeability of enalapril (5 μM) in Caco-2 cells increased significantly, by 30-133%, over the range of juice concentrations tested (6.25-50%). Cellular accumulation of enalapril at 1 h increased by 39-87%, respectively, while that of enalaprilat decreased by 12-32%. Enalapril hydrolysis in both S9 fractions was inhibited by <20% up to 40% juice. The permeability of lovastatin (5 μM) increased in the presence of GFJ, by 40% and 22% at 6.25% and 12.5% juice, respectively, then decreased at the higher juice strengths (25% and 50%), possibly due to binding of drug to GFJ pulp in the apical compartment. Cellular accumulation of lovastatin at 1 h decreased by 5-42%, and lovastatin acid formation decreased by 29-80%, over the range of juice concentrations tested (6.25-50%). Lovastatin hydrolysis was reduced by ~50% in human intestinal S9 fractions up to 40% juice. Collectively, these *in vitro* observations suggested that GFJ inhibited enteric esterase activity, leading to increased prodrug stability.

When GFJ concentrate (diluted 1:3) was administered orally to rats, before intravenous administration of enalapril or lovastatin (2 mg/kg), clearance and half-life of both prodrugs were unchanged relative to water, indicating that GFJ had no effect on hepatic esterase/Cyp3a activity [105]. After oral administration of enalapril (10 mg/kg) with water or GFJ concentrate (diluted 1:3, 1:2, and undiluted), mean AUC of enalaprilat was increased, by 65, 70, and 16%, respectively, relative to water; prodrug was not measured. The decreased exposure at the higher strength was attributed to binding of drug to GFJ pulp. These results were consistent with observations with the esterase inhibitor, bis-*p*-nitrophenylphosphate. After oral administration of lovastatin (10 mg/kg) with water or GFJ concentrate (diluted 1:3, 1:2, and undiluted), mean AUC of lovastatin acid was increased by 279, 157, and 170%, respectively, relative to water; prodrug was not measured. Since lovastatin is a substrate for CYP3A/Cyp3a, the contribution of esterase inhibition was differentiated by measuring Cyp3a- and esterase-mediated metabolites in portal vein-cannulated rats pre-treated with GFJ (diluted 1:3). Both Cyp3a and esterase inhibition by GFJ led to similar increases in exposure to lovastatin and the active acid, as well as unchanged CYP3A-mediated metabolites, suggesting equal contribution by Cyp3a and esterase to the interaction. Taken together, these *in vivo* observations were consistent with enteric esterase inhibition by GFJ, leading to increased prodrug stability in enterocytes and higher exposure to active metabolite *via* hydrolysis in plasma.

A follow-up *in vitro* study by the same investigators examined the esterase inhibition potential of 10 GFJ components toward *p*-nitrophenylacetate (PNPA) hydrolysis in human liver microsomes [106]. The flavonoids kaempferol, quercetin, and naringenin showed potent inhibition of PNPA hydrolysis, with IC_{50} values of 62, 43, and 30 μM, respectively. The effect of kaempferol and naringenin on esterase-mediated hydrolysis of enalapril and lovastatin also were evaluated in Caco-2 cells and in rats. Compared to control (buffer), the absorptive permeability coefficient of enalapril (20 μM) in Caco-2 cells was increased with kaempferol and naringenin (each at 250 μM) by 80% and ~200%, respectively, whereas that of lovastatin (20 μM) was increased by ~65% with both flavonoids. Intracellular concentrations of enalaprilat and lovastatin acid decreased by ~60% and 46-70%, respectively, consistent with inhibition of esterase activity. Oral

administration of enalapril and lovastatin (both at 10 mg/kg) with naringenin (10 mg/kg) to rats increased active metabolite AUCs significantly, by 38% and 288%, respectively, relative to water. Similarly, oral administration with kaempferol (10 mg/kg) increased metabolite AUCs by 109 and 246%, respectively. Finally, in portal vein-cannulated rats, kaempferol (10 mg/kg) increased portal plasma exposure to lovastatin and lovastatin acid by 154% and 113%, respectively. Collectively, these observations suggested some flavonoids as potential candidate enteric esterase inhibitors in GFJ. However, more studies are needed to determine the clinical utility, as well as other causative ingredients, of this new type of drug-GFJ interaction.

Sulfotransferase

Conjugative enzymes generally increase hydrophilicity, facilitating elimination of endogenous substrates and xenobiotics [107]. Sulfotransferases (SULTs) catalyze the conjugation of 3′-phosphoadenosine 5′-phosphosulfate with a number of endogenous low molecular weight compounds (*e.g.*, steroids, catecholamines) and xenobiotics [108]. Three human SULT subfamilies have been identified, with at least 13 distinct members distributed in liver, brain, intestine, lung, kidney, and other tissues [109]. Some fruit juices have been shown to inhibit two members of the SULT1 family *in vitro*: SULT1A1 and SULT1A3, the latter of which is expressed only in extrahepatic tissues, including the intestine.

Grapefruit Juice. SULT1A1 and SULT1A3 inactivate β_2-adrenergic agonists in the liver and intestine, respectively [110]. The bronchodilators albuterol and terbutaline undergo extensive first-pass metabolism in both organs to sulfate conjugates [111]. An *in vitro* study using human recombinant SULT1A1 and SULT1A3 investigated the inhibitory effects of GFJ, orange juice, and various teas on SULT activity, as measured by *p*-nitrophenol and dopamine sulfation for SULT1A1 and SULT1A3, respectively [112]. GFJ, at a concentration of 10% (v/v), inhibited SULT1A1 and SULT1A3 by >90% and 50%, respectively, relative to control. Specific juice components also were tested and included naringin, naringenin, quercetin, bergamottin, and 6′,7′-dihydroxybergamottin. Quercetin was the most potent, inhibiting by >90% (SULT1A1) and 50% (SULTA3), at a concentration of 10 μM.

Orange Juice. Orange juice was tested in the same manner as GFJ in the aforementioned *in vitro* study [112]. As observed with GFJ, orange juice (10%, v/v) inhibited both SULTs, by >95% (SULT1A1) and 20% (SULT1A3). The orange juice components, tangeretin and nobiletin (both at 10 μM), were the most potent single components, inhibiting SULT1A1 almost completely and SULT1A3 by ~20%. As with GFJ, whether or not these observations translate to the clinic merits further investigation.

Pomegranate Juice. The effect of pomegranate juice on sulfoconjugation was evaluated in Caco-2 cells [113]. The extent of inhibition of 1-naphthol sulfation by pomegranate juice was both concentration- and cell culture time-dependent. At the highest concentration tested (5%, v/v), the juice had no effect on SULT1A1 and SULT1A3 expression for up to 24 hours. Punicalagin, the most abundant polyphenol in pomegranate juice, was isolated and shown to inhibit sulfoconjugation in the cells, with an IC_{50} of 45 μM. Clinical significance of these *in vitro* observations has not been reported.

Efflux Transport Proteins

The influence of efflux transporters is considered integral to drug disposition [114]. Similar to inhibition of enzymes, inhibition of efflux transporters can lead to altered systemic and local drug concentrations. The most well-characterized efflux transporter, P-gp, shares tissue distribution and substrate specificity with many CYPs, especially CYP3A [115]. Due to the apical (lumenal) location on membranes of enterocytes, P-gp functions to extrude substrates back into the intestinal lumen, lowering systemic drug concentrations. Thus, as with enteric CYP3A, inhibition of enteric P-gp would be expected to enhance systemic drug exposure.

Grapefruit Juice. Whether or not GFJ modulates intestinal P-gp activity remains controversial [110]. One reason for the inconsistency is use of P-gp substrates that also are CYP3A substrates [116]. The contribution by P-gp and CYP3A is difficult to distinguish. Cyclosporine, a commonly used immunosuppressant with a narrow therapeutic window, is one such dual CYP3A4/P-gp substrate shown to interact with GFJ [117]. The increase in cyclosporine AUC ranges from 20 to 60%, relative to water or orange juice [47]. To assess whether furanocoumarins mediate the cyclosporine-GFJ interaction, a randomized crossover study involving 18 healthy volunteers compared the effects of GFJ, a "furanocoumarin-free" GFJ (prepared from the GFJ), and orange juice (control) on oral cyclosporine PK [63]. Median dose-corrected cyclosporine AUC with GFJ was significantly higher (by ~38%) than that with orange juice. In contrast, relative to orange juice, furanocoumarin-free GFJ had no consistent effect, with a median concentration-time profile that was indistinguishable from that with orange juice. Complementary *in vitro* studies with Caco-2 cell monolayers showed that, relative to vehicle, diluted extracts derived from GFJ and orange juice, as well as two purified furanocoumarins (bergamottin and 6′,7′-dihydroxybergamottin), partially increased cyclosporine apical-to-basolateral translocation, whereas the furanocoumarin-free GFJ extract had no effect. These observations supported furanocoumarins as candidate P-gp inhibitors in GFJ. Furanocoumarins were concluded to mediate, at least partially, the cyclosporine-GFJ interaction *in vivo* through inhibition of enteric CYP3A and possibly enteric P-gp.

Recent studies in rats evaluated potential interactions with the anti-gout agent colchicine and antiemetic domperidone, both of which are dual CYP3A/P-gp substrates. The effect of a GFJ concentrate on colchicine intestinal permeability was evaluated in Caco-2 cell monolayers and in the *in situ* rat intestinal perfusion model [118]. With Caco-2 cells, at the highest concentration of GFJ tested (10%, v/v), colchicine apical-to-basolateral translocation was increased by 75%, and basolateral-to-apical translocation was decreased by 45%, relative to control (transport buffer). In addition, GFJ (10%, v/v) increased colchicine ileal and jejunal permeability by 2- and 1.5-fold, respectively, in the *in situ* perfused intestine. These data were consistent with inhibition of enteric P-gp by GFJ. The effect of a commercially available GFJ extract was evaluated on domperidone exposure in rats [119]. Domperidone (10 mg/kg) was administered orally, two hours after GFJ extract (2 mL/kg). The sum of partial AUCs (0-0.25 h, 0-2 h, 4-8 h) with GFJ extract was 16% greater than that with water, albeit the difference was not significant ($p>0.05$). As with cyclosporine, interpretation of the underlying mechanism of the colchicine- and domperidone-GFJ interactions is confounded by the dual CYP3A/P-gp nature of these substrates. However, unlike cyclosporine, clinical relevance has not been established.

The inconclusive results of studies utilizing dual CYP3A/P-gp substrates could be resolved using P-gp substrates that undergo negligible metabolism. Although GFJ has been shown to inhibit

translocation of such substrates *in vitro*, observations have not translated to the clinic. For example, GFJ had a negligible effect on systemic exposure to digoxin, as evidenced by a <10% increase in mean AUC_{0-24} relative to water [120]. Other minimally metabolized substrates, including the antihistamine fexofenadine and the β-blockers talinolol and celiprolol, also have been tested. Unexpectedly, healthy volunteer studies showed a significant *decrease* in mean AUC of these three drugs when taken with GFJ, by 13-63% relative to water [59, 121, 122], prompting investigations of this newly identified mechanism underlying drug-fruit juice interactions (see **Uptake Transport Proteins**).

Orange Juice. Pravastatin, which undergoes minimal metabolism, is a substrate for P-gp and two other apically-located efflux transporters, multi-drug resistance-associated protein 2 (MRP2) and breast cancer-resistance protein, as well uptake transporters (organic anion transporting polypeptides) [123]. A clinical study involving 14 healthy volunteers given a single dose of pravastatin (10 mg) and multiple glasses of commercially available orange juice (reconstituted from concentrate) given over 195 min (total volume, 800 mL) showed a 50% increase in mean AUC of pravastatin relative to water [124]. The authors speculated upregulation of pravastatin absorption in the intestine by orange juice, as a similar study in rats showed an increase in intestinal Oatp1 and Oatp2 mRNA and protein expression. Net inhibition of efflux by orange juice also may explain this interaction. Naringin and some polymethoxyflavones have been shown to inhibit P-gp and MRP2 *in vitro* and may represent candidate causative ingredients [125, 126].

Uptake Transport Proteins

Organic anion transporting polypeptides (OATPs) are transmembrane transporters that facilitate uptake of a number of endogenous compounds and drugs [127]. These transporters are gaining attention as important determinants of drug disposition [128]. The human OATP family consists of 11 members, with OATP1A2, OATP1B1, OATP1B3 and OATP2B1 as the most characterized [129]. Of these, OATP1A2 and OATP2B1 have been reported to be expressed on apical membranes of enterocytes [130].

Grapefruit Juice. The initial clinical study examining effects of fruit juices, including GFJ, on enteric P-gp activity using fexofenadine as a probe substrate showed an unforeseen mean decrease in fexofenadine AUC, by 63% relative to water (see **Efflux Transport Proteins**). Mean elimination half-life was unchanged. This atypical interaction was attributed to inhibition of an apically located intestinal uptake transporter [59]. Subsequent clinical and *in vitro* studies substantiated GFJ as an inhibitor of enteric OATP activity [131-134]. Reduced exposure could lead to reduced effect. Indeed, the package insert for fexofenadine (Allegra®) notes that the size of histamine-induced skin wheal and flare was significantly larger when fexofenadine was taken with GFJ (or orange juice) than when taken with water [135]. Based on these PK and PD outcomes, the manufacturer recommends taking fexofenadine with water. In addition to fexofenadine, GFJ has been shown to decrease mean AUC of other OATP substrates, including talinolol [121], celiprolol [122], acebutolol [136], etoposide [137], and L-thyroxine [138], by 11 to 56% relative to water. Only two of these studies (celiprolol and L-thyroxine) assessed PD outcomes and reported no effect, albeit in healthy volunteers. Clinical significance for the remaining substrates has not been established. Nevertheless, inhibition of enteric OATPs is recognized as an additional mechanism of altered drug disposition by GFJ. That is, GFJ can

decrease, significantly, systemic exposure to OATP substrates, with a consequent potential for reduced efficacy. This relatively new type of mechanism is discussed in detail in two recent reviews [139, 140].

OATP1A2 and the flavonoid, naringin, have been proposed as the major transporter and causative ingredient involved in the interaction between GFJ and fexofenadine, as well as talinolol. *In vitro* studies with OATP-transfected human epithelial cervical cancer (HeLa) cells supported a role for OATP1A2 in uptake of both drugs [59, 61, 141]. One *in vitro* study assessed the uptake activity of several OATPs, and showed OATP1A2 as the only enteric OATP capable of taking up fexofenadine [133]. A clinical study investigating the impact of GFJ on intestinal transporter expression showed no difference in OATP1A2 protein (and P-gp) expression between GFJ and water in duodenal biopsies obtained from healthy volunteers, suggesting GFJ may not destroy transport proteins *via* mechanism-based inhibition [133]. That is, the mechanism of inhibition of enteric transporter activity by GFJ may differ from that of enteric CYP3A activity. Unlike OATP2B1-transfected HeLa cells, a separate study with OATP2B1-transfected human embryonic kidney (HEK) 293 cells demonstrated fexofenadine as a substrate for OATP2B1 [142]. GFJ and components (including naringin) have been shown to inhibit uptake of the OATP substrate estrone-3-sulfate in OATP2B1-transfected HEK293 cells, by up to 80% [134], but additional studies are needed to determine whether GFJ/components inhibit OATP2B1-mediated uptake of fexofenadine, as well as to clarify differences in fexofenadine uptake between transfected cell lines.

Naringin has been implicated as a major causative enteric OATP inhibitor in GFJ. Healthy volunteers (n=12) were given fexofenadine (120 mg) with GFJ (300 mL), an aqueous solution of naringin at the same concentration as that in GFJ (1200 μM), or water [61]. Relative to water, GFJ and naringin decreased fexofenadine mean AUC by 42% and 22%, respectively. The authors concluded that naringin most likely inhibited enteric OATP1A2, resulting in decreased fexofenadine bioavailability. The 50% difference in fexofenadine AUC between GFJ and naringin suggested other ingredients contribute to the fexofenadine-GFJ interaction.

A recent *in vitro* study involving the leukotriene receptor antagonist, montelukast, and a clinical trial involving the renin-inhibiting antihypertensive agent, aliskiren, added two potential drugs to the growing list of enteric OATP-mediated drug-GFJ interactions [143, 71]. *In vitro* studies with Caco-2 cells and OATP2B1-transfected Madin-Darby canine kidney cells demonstrated that montelukast undergoes carrier-mediated transport by OATP2B1. GFJ at 5% and 10% (v/v), and orange juice at 10%, reduced montelukast permeability significantly ($p<0.05$), by ~30% relative to control (buffer). Clinical relevance of these interactions has not been examined. Aliskiren is a substrate for OATP2B1, as well as CYP3A and P-gp. Healthy volunteers (n = 11) were administered single-strength GFJ (200 mL) three times daily for 5 days, and aliskiren (150 mg) was given on the third day. Relative to water, GFJ significantly reduced mean aliskiren AUC and C_{max}, by 61% and 81%, respectively. Mean elimination half-life remained essentially unchanged. Net inhibition of enteric OATP-mediated uptake by GFJ could account for the reduced exposure. Other potential mechanisms included a physicochemical interaction between GFJ and aliskiren or an alteration of physiologic conditions in the gut by GFJ. Follow-up *in vitro* and clinical studies are needed to clarify the role of OATP in the aliskiren-GFJ interaction, as well as effect on PD outcomes.

Orange Juice. Orange juice contains trace amounts of furanocoumarins and has minimal enteric CYP3A inhibitory effect [126, 144]. As such, orange juice has been used as a control juice, rather than water, in some clinical studies. However, orange juice has been shown to reduce systemic exposure, significantly (by 22-83%), to fexofenadine [59], atenolol [145], and celiprolol [146]. Decreased mean AUC (up to 38%) also has been observed for the fluoroquinolones ciprofloxacin [147] and levofloxacin [148] with calcium-fortified and non-fortified orange juice. Any or all of these interactions could involve inhibition of enteric OATP by orange juice, as fexofenadine, levofloxacin, and celiprolol, have been shown to be substrates for OATP *in vitro* [59, 149, 150].

Hesperidin, a major component of orange juice, is a flavonoid glycoside with a molecular structure similar to that of naringin [151]. Hesperidin has been shown to inhibit OATP1A2-mediated uptake of fexofenadine *in vitro*, with an IC_{50} of 2.7 μM [61], similar to that of naringin (3.6 μM). However, hesperidin produced only 60% maximum inhibition at the highest tested concentration (100 μM). A study in rats duodenally administered celiprolol (5 mg/kg) and orange juice or aqueous solution of hesperidin (208 μg/mL or 340 μM, the same concentration as that in the orange juice) showed a mean AUC decrease of 75% and 78%, respectively, relative to water. The AUC in the hesperidin group was not significantly different than that of the orange juice group, suggesting hesperidin contributes to the celiprolol-orange juice interaction. Studies with other OATP substrates would clarify the *in vivo* significance of hesperidin.

Pomelo Juice. The previously mentioned clinical study of six healthy male volunteers showed a significant decrease (by ~60%) in mean AUC and C_{max} of sildenafil after ingestion of a 240-mL glass of fresh-squeezed pomelo juice [77] (see **Cytochrome P450 3A**). One possible explanation was inhibition of intestinal uptake (*e.g.*, by OATP) of sildenafil by the juice. No follow-up studies examining sildenafil as an OATP substrate have been reported.

Apple Juice. The effect of apple juice on fexofenadine uptake also was evaluated in the initial fexofenadine-GFJ interaction study [59]. The OATP-mediated uptake of [^{14}C]fexofenadine was examined in the presence and absence of increasing concentrations (0-5%, v/v) of apple juice in OATP1A2-transfected HeLa cells. The highest concentration of juice inhibited activity by >85% relative to water. Clinical study results also were significant, as apple juice decreased mean AUC of fexofenadine by ~70% compared to water. To the authors' knowledge, no follow-up *in vitro* and clinical studies involving apple juice *per se* have been published. However, a recent *in vitro* study investigated the effect of three flavonoids (apigenin, kaempferol, quercetin) on OATP activity in OATP1A2- and OATP2B1-transfected HEK293 cells using fexofenadine and bromosulfophthalein as substrates [152]. Quercetin, known to be present in apples (*Malus domestica*) and apple juice [153], inhibited OATP1A2-mediated fexofenadine uptake, with an IC_{50} of 13 μM. Quercetin also inhibited OATP1A2- and OATP2B1-mediated bromosulfophthalein uptake, with K_i values of 22 and 8.7 μM, respectively. Further studies are needed to determine if quercetin is a major causative ingredient in apple juice *in vivo*.

DISCORDANCE BETWEEN *IN VITRO* AND CLINICAL STUDIES

Although a number of fruit juices have been shown to inhibit several intestinal CYPs and transporters *in vitro*, some of the interactions have not translated to the clinic. Fruit juices clearly inhibit intestinal metabolism and transport, but the magnitude of change in C_{max} and/or AUC

often is insignificant, unpredictable, and highly variable, which cannot be explained adequately. These *in vitro-in vivo* discordances may be due to a lack of sufficient information to determine a positive interaction. A deficiency common to most drug-fruit juice interaction studies is a limited or non-existent chemical description of the juice. Although several reasons account for discrepancies between *in vitro* and clinical studies [24, 154], one explanation is that the concentration of inhibitors in the juice might not be sufficient to inhibit metabolism/transport *in vivo*. The sources and complexity of a plant's chemical constituents are underappreciated. Concentrations of bioactive compounds in a natural food product vary depending on ecological conditions, manufacturing process, storage conditions, and a host of other environmental factors [155]. Thus, testing a random juice product *in vitro* and *in vivo* without understanding the chemical makeup provides no basis for comparison between studies. One of the most fundamental solutions to establishing meaningful physiological dose-response relationships for dietary substances is to characterize the product prior to use.

Few *in vivo* drug-fruit juice interaction studies reported concentrations of bioactive constituents in the clinical test juice. Since the establishment of furanocoumarins as unequivocal mediators of enteric CYP3A-based interactions in 2006 [53], only a handful of clinical studies involving CYP3A substrates reported furanocoumarin content in the test juice (Table 2). Furanocoumarins have been studied to the extent that they can be considered 'marker' compounds, defined as compounds unique to a dietary substance [156]. Characterization of a given juice in terms of furanocoumarin content could be used to predict the likelihood and magnitude of an interaction. For example, likelihood of an interaction can be predicted by the presence of furanocoumarins, and effect size can be correlated to the amount of marker compound(s). Between-study comparisons also can be made. Indeed, 6′7′-dihydroxybergamottin was used in a recent PK modeling study investigating the impact of CYP3A-based inhibition on drug disposition [157]. In addition to grapefruit and related citrus juices, furanocoumarins are present in substantial amounts in umbelliferous vegetables (*e.g.*, parsnips, celery, parsley) and are not destroyed by normal cooking procedures [158]. Furanocoumarins also are present in Kampo extract medicines, which originated in Japan and are used widely in Asia [159]. A similar strategy could be applied to predict likelihood and magnitude of interactions between these foods/natural medicines and traditional medications.

APPLICATIONS: NEW TWISTS ON OLD INTERACTIONS

Mechanisms and causative ingredients underlying enteric CYP3A inhibition by GFJ have been studied for more than two decades [160]. The information gained has been used by different groups to their advantage. For example, the potential for GFJ to increase systemic drug concentrations, and consequent PD effect, of certain opiates is a widely discussed topic among recreational users in online forums [161, 162]. The scientific community also has attempted to exploit enteric CYP3A inhibition for pharmacoeconomic and therapeutic purposes. Intentional manipulation of enteric CYP3A by GFJ and individual components is of particular interest in the cancer treatment and organ transplantation areas [137, 163-166]. For example, inhibition of enteric CYP3A by GFJ could improve oral bioavailability of some agents without GFJ exerting additional adverse effects. In addition, the cost and side effect severity of these multi-drug and toxic regimens could be reduced through dose and/or dosing frequency reduction by coadministration with GFJ, possibly improving adherence. However, several drugs in these therapeutic classes have a narrow therapeutic range and require close monitoring. Without

thorough characterization of the juice product, these practices are at best ineffective, and at worst, place the patient at risk for unfavorable outcomes.

Table 2: Clinical drug-citrus juice interaction studies in which candidate causative ingredients in the test juice were quantified

Juice	Drug Tested	Confirmed or Suspected Active Constituent	Constituent Concentration in Test Juice(s) (μM)	Reference
Grapefruit	Fexofenadine	Naringin	1200	[61]
	Cyclosporine	GFJ/FC-free GFJ/OJ*	11.5/0.08/ND	[63]
		DHB	9.5/0.03/ND	
		BG	149/104/ND	
		Narirutin	440/331/ND	
		Naringin	8/5/ND	
		Hesperidin	15/8/ND	
		Neohesperidin	15/2/ND	
		Didymin	30/9/ND	
		Poncirin	0.56/0.01/7.66	
		Nobiletin	0.15/0.02/1.95	
		Tangeretin		
	Dextromethorphan	DHB	37	[64]
		BG	27	
		Naringin	850	
		Naringenin	0.82	
	Sunitinib	DHB	2.7, 5.7†	
		BG	33, 24†	[69]
Pomelo	Felodipine	Guanximiyou/Changshanhuyou§		[76]
		DHB	1.3/ND	
		BG	8.2/ND	
		Paradisin A	1/ND	
		Paradisin B	0.1/ND	
		Paradisin C	3.5/ND	

*Units are ppm
†A second lot of the same brand was used for the last two patients due to expiration date.
§Two varieties of pomelo fruit
DHB, 6′,7′-dihydroxybergamottin; BG, bergamottin; FC, furanocoumarin; OJ, orange juice; NS, not significant; ND, not detected

An *in vivo* study of wild-type and humanized CYP3A4 transgenic mice orally administered the anticancer agent, erlotinib, and BAS 100, a spiro-ortho-ester mechanism-based CYP3A4 inhibitor isolated from GFJ, demonstrated a 2.1-fold increase in erlotinib AUC, relative to control (saline) [163]. Results illustrated the potential of BAS 100 to “boost” systemic drug exposure, and decrease associated variability, of erlotinib in cancer patients. The erlotinib study is one of the first attempts to test the strategy of deliberate inhibition of intestinal first-pass metabolism.

A clinical trial investigating the effect of GFJ on sirolimus PK in advanced solid tumor patients is ongoing [167]. Initial results showed no effect, possibly due to insufficient furanocoumarin content in the GFJ product selected [168]. This approach is unsettling because GFJ was chosen as a ‘boosting’ agent for a narrow therapeutic index drug but was not characterized before administration. A more ‘potent’ GFJ containing inhibitory concentrations of furanocoumarins (not reported) given subsequently to the subjects significantly increased plasma concentrations of sirolimus, by up to 400% relative to water [164]. A daily glass of GFJ (240 mL) was projected to lower sirolimus costs by 50% [169]. The same investigators have suggested ‘GFJ boosting’ to reduce dose and cost for the tyrosine kinase inhibitor, lapatinib [165]. One 250-mg lapatinib tablet, accompanied by food and/or GFJ, was speculated to increase systemic exposure

comparable to that of five 250-mg tablets on an empty stomach, resulting in a total cost savings of 80%.

The strategy of using GFJ to lower drug costs also has been considered in the management of immunosuppression. Approximately 20 years ago, it was proposed that a compound which inhibits pre-systemic metabolism of cyclosporine without causing systemic effects could have clinical value [170, 171]. GFJ would seem an ideal candidate since it has been shown to increase cyclosporine exposure [172]. However, the efficacy and pharmacoeconomic impact of combining cyclosporine and GFJ have not been determined. A prospective clinical study evaluated the effect of two commercially available GFJ products on tacrolimus PK in liver transplant recipients [173]. After administration of GFJ (250 mL twice daily) for one week, mean trough tacrolimus concentration was enhanced significantly, by ~10 ng/mL compared to baseline. The dose of tacrolimus was decreased by ~2 mg per day, which amounted to a savings in drug costs of ~$9 per day. The safety and pharmacoeconomic benefit of a GFJ boosting strategy have not been evaluated sufficiently to change disease management. As with the anti-cancer agents, promising conclusions are unwarranted due to insufficient data on the PK-PD relationship between the causative ingredients in GFJ (*e.g.*, furanocoumarins) and tacrolimus, as well as the large interindividual variability in response, making therapeutic outcomes virtually unpredictable.

It seems tempting to take advantage of the effects of a natural product like GFJ to boost systemic drug exposure and decrease inter-/intraindividual variability in PK, and ideally PD, outcomes. The dose and dosing schedule of certain drugs could be reduced to lower drug costs and improve patient compliance. However, further research is required on the mechanisms of action, causative ingredients, and PK-PD relationship with respect to individual juice components and the drug of interest. Given the possibility of using GFJ and/or individual constituents as a ‘drug-sparing agent,’ a standardized approach to investigating interaction potential is imperative. Early evaluation of CYP and transporter inhibition properties of new chemical entities is routine during drug development. A similar approach could be adopted for dietary substances. However, information providing a systematic approach for the study, prediction, and management of drug-dietary substance interactions is lacking. Ideal management approaches would be those developed based on well-designed *in vitro* studies. Information gained from rigorous *in vitro* studies, combined with that gained from *in silico* methods, could optimize clinical study design and clarify the clinical significance of an interaction. Robust PK/PD models could then be used to determine potential risks of long-term inhibition of intestinal metabolism/transport by a given dietary substance on pharmacotherapeutic outcomes.

CONSIDERATIONS FOR IMPROVED RESEARCH PRACTICES

Dietary substances in the United States, which include supplements/herbal remedies/nutraceuticals, are regulated under the same framework as foods, separate from the regulation of drugs. Individuals representing multiple sectors (science, clinical practice, public) have argued both for and against this policy, which is unlikely to change in the foreseeable future. As such, it is unrealistic to expect the U.S. Food and Drug Administration (FDA) to require high-quality scientific evidence from the relevant industries [174]. It also is unlikely that the FDA will require more extensive drug-dietary substance interaction studies other than those recommended. However, legislation need not be passed to undertake sound scientific research.

Several approaches can be adopted for rigorous evaluation of potential drug-dietary substance interactions. Practices regarding peer review of the drug interaction liability of a dietary substance should be the same as those for a drug, particularly with respect to reproducibility. If the dietary substance is not described in detail, then other investigators will be unable to reproduce one or more facets of the study.

Since dietary substances contain multiple, often unknown, bioactive ingredients that vary in composition between batches and manufacturers, characterization of causative ingredients and mechanisms of action is essential. Identification of components responsible for these interactions can be challenging. Generally, biologic action is not determined by a single active compound. A set of 'marker' compounds that can be applied for definitive authentication of the test material would serve as an indicator of quality and potency. The selected markers should be unique to the selected species and represent health-relevant principles [156]. The identity of constituent(s) should be confirmed initially by *in vitro* methods that screen for potential interactions. Such experiments provide mechanistic information about inhibitory capacities, as well as specific enzymes and/or transporters involved.

Reporting of the characterization of dietary substances used in clinical trials must be improved. Many clinical studies lack basic information about the test material. Considering the requirements for a drug investigated clinically, the disconnect becomes obvious. A substance derived from a "natural source" does not imply that the rigors of reproducibility should be abandoned. At minimum, for commercially available products, the brand name, manufacturer, lot number, ingredients, preparation directions, manufacturing process, and origins of growth and production should be stated. For freshly prepared test material, scientific name, quantity, plant part used, site of collection, preparation procedures, and storage conditions should be documented [175-177]. If a marker compound has been identified or suspected, quantitative analysis by analytical chemistry techniques should be conducted to determine the presence and/or quantities of relevant constituents. Since administration of 'standardized' fruit juices is not possible, it would be more realistic to quantify a particular known/suspected component, or group of components, prior to use. This practice would enable some degree of between-study comparison.

Stringent methods for evaluating potential drug-dietary substance interactions are critical as new products enter the market. However, the aforementioned tasks do not rely solely on one discipline. Rigorous investigations of complex botanical products require collaboration from many areas, including clinical pharmacology, pharmacognosy/natural products chemistry, and botany [178]. Specific botanical expertise, combined with knowledge of appropriate assays and other experimental tools for testing compounds, would improve greatly the deficiency in the characterization of natural products used in clinical trials. A multidisciplinary, translational research approach is necessary to explain fully these relatively unexplored types of drug interactions.

SUMMARY AND PERSPECTIVE

Interactions between medications and dietary substances, as foods or supplements, remain a relatively understudied, likely underreported, and generally misunderstood area of pharmacotherapy. Growing impatience over the slow introduction of innovative drugs on the

market, combined with rising health care costs, has contributed to the ever-increasing obsession by consumers with quick-fix, “all-natural” remedies. Concurrent with the increasing use of dietary supplements and so-called ‘superfoods’, including fruit juices, is the upward trend of polypharmacy, all hindering optimal therapeutic outcomes. Discovery of the effect of GFJ on felodipine PK and PD launched renewed interest in the study of drug-dietary substance interactions. Although inhibition of enteric CYP3A has been studied extensively, questions still remain. Additional mechanisms involving inhibition of enteric active uptake transporters have been identified. *In vitro* studies have added to the list of drugs that may interact with dietary substances *in vivo*, but well-designed, proof-of-concept clinical studies are limited. Numerous other potential drug-dietary interactions are anticipated to emerge as new supplements and different types of foods (*e.g.*, organic, functional, genetically modified, fortified) are created. The complexity of dietary substances and lack of adequate characterization preclude between-study comparisons, as well as accurate predictions of drug interaction liability. Ongoing challenges involve systematic investigations of underlying mechanisms, causative ingredients, and PK-PD relationships with respect to individual juice components and drugs of interest. The ultimate goal is to develop common practice guidelines to provide a consistent approach in managing drug-dietary substance interactions appropriately.

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ABBREVIATIONS

DDIs	drug-drug interactions
PK	pharmacokinetics
PD	pharmacodynamics
CYP	cytochrome P450
GFJ	grapefruit juice
AUC	area under the curve
FC	furanocoumarin
MOM	3-methoxymorphinan
ER	extended release
tid	three times daily
d	days
NSP	not specified
bid	two times daily
qd	daily
C_{max}	maximum concentration
NS	not significant
CBJ	cranberry juice
UTI	urinary tract infection
P-gp	P-glycoprotein
INR	International Normalized Ratio
PNPA	*p*-nitrophenylacetate
IC_{50}	half maximal inhibitory concentration
SULTs	sulfotransferases

MRP2	multi-drug resistance-associated protein 2
OATPs	organic anion transporting polypeptides
HEK	human embryonic kidney
K_i	inhibition constant
DHB	6′,7′-dihydroxybergamottin
BG	bergamottin
FC	furanocoumarin
OJ	orange juice
ND	not detected
FDA	Food and Drug Administration

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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