Clinical relevance of the small intestine as an organ of drug elimination: drug-fruit juice interactions

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Abstract:
Most drugs are taken orally. For those intended to act systemically, a significant fraction of the dose can be eliminated during its first passage through a sequence of organs before entry into the general circulation. For some drugs, the degree of first-pass elimination can be large enough such that oral bioavailability is significantly reduced, with the consequent potential for a reduced clinical response. Of these first-pass eliminating organs, the small intestine and liver are the most commonly implicated, in part because they express the highest levels of drug-metabolizing enzymes. For several drugs whose major route of elimination occurs via CYP3A-mediated metabolism, the extent of first-pass metabolism in the small intestine can rival that in the liver. As such, alterations in enteric CYP3A activity alone can significantly influence oral bioavailability. The most extensively studied xenobiotic shown to inhibit only enteric CYP3A is grapefruit juice, the consequences of which can be clinically significant. Although much information has been gained regarding the grapefruit juice effect, progress in the relatively understudied area of drug-diet interactions continues to be sluggish and reactive. In stark contrast, the potential for drug-drug interactions involving any new therapeutic agent must be evaluated, prospectively, before market introduction. To prospectively elucidate mechanisms underlying drug-diet interactions, a multidisciplinary, translational research approach is required, which capitalizes on the collective expertise of drug metabolism scientists and natural products chemists. Such an approach would allow proper between-study comparisons, and ultimately provide conclusive information as to whether specific dietary substances can be taken safely with certain medications.
Keywords: cranberry, CYP3A, drug—diet interactions, drug—fruit juice interactions, first-pass metabolism, furanocoumarins, grapefruit, natural product, oral bioavailability, pomegranate

Article:
1. Introduction
The oral route continues to serve as the most popular, convenient and generally safest means of drug administration. However, this route is not necessarily the most efficient, particularly for systemically acting drugs, due to the many anatomical and physiological barriers drugs encounter from the time of ingestion until the time of entry into the general circulation. As a consequence, before the drug reaches the circulation and exerts its effects in the target tissue(s), significant loss of the original dose can occur as the drug passes, for the first time, through the following sequence of organs: the gastrointestinal (GI) tract, the liver and the cardiopulmonary system (Figure 1). Processes that can lead to significant loss of active drug include incomplete release from the dosage form, degradation in the GI lumen, poor permeation across the GI epithelial barrier, active efflux into the GI lumen, biliary excretion, and metabolism. Of these processes, only metabolism can occur in all of the aforementioned organs.

Metabolism represents the primary means by which the body eliminates xenobiotics, including the majority of drugs. Enzymatic alteration of the drug usually produces Inactive metabolItes with increased water solubility to facilitate excretion. For many drugs, the extent of conversion to inactive metabolites can be large enough such that circulating levels of active drug are significantly reduced, which in turn can lead to a significant decrease in

pharmacological activity and a reduced clinical response. Drugs with a narrow therapeutic window that undergo extensive presystemic metabolism are especially vulnerable to a reduced clinical response. Of the first-pass organs of elimination, the liver is the most commonly implicated, in part because it contains the highest levels of drug-metabolizing enzymes. Next to the liver, the intestinal mucosa undoubtedly represents the most important extrahepatic site of drug biotransformation 11]. Several of the drug-metabolizing enzymes expressed In hepatocytes are also expressed in the epithelial cells that line the small intestinal mucosa (enterocytes), including both phase I (oxidative and reductive) and phase II (conjugative) enzymes. Of the phase I enzymes, the CYPs are the most prominent, with the CYP3A subfamily, composed primarily of CYP3A4 and the polymorphic CYP3A5 in adults 12,31, representIng the largest portion (80%) of total CYP content in the proximal small intestine 141. So far, only CYP3A has been shown conclusively to have clinical relevance, as is discussed in greater detail in later sections of this review. Other phase I enzymes expressed in the human small intestine include carboxylesterases and epoxide hydrolases; the former are believed to contribute to the first-pass conversion of the prodrug irinotecan to its active chemotherapeutic metabolite, SN-38 151. Enteric phase II enzymes include UDP-glucuronosyl transferases (UGTs), sulfotransferases (SULTs), N-acetyl transferases and glutathione S-transferases. Of these enzymes, the UGTs and SULTs are the most commonly implicated in the metabolism of drugs. Each of the various UGT and SULT subfamilies are described in detail in several excellent reviews 16-9J.

2. Clinical relevance of intestinal metabolism

The small intestine represents the first in the serIes of organs that drugs encounter when taken orally. As such, for drugs that are absorbed by the transcellular route, the opportunity exists for first-pass metabolism, the extent of whIch can have clinical ramifications. Of the numerous drug-metabolizing enzymes expressed in the intestinal mucosa, the CYP3A subfamily Is the most extensively studied, due in part to its established role as a major contributor to the interindividual variation in drug oral bioavailability, as well as a major site for drug—drug, and especially drug—diet, interactions.

2.1 Influence on oral bioavailability

The oral bioavailability (F_{oral}) of a drug refers to the fraction of the oral dose that reaches the systemic circulation In the unchanged form. Because metabolism is frequently the major cause of first-pass drug elimination, F_{orai} is often used as a measure of the extent of first-pass metabolism 1101. Foral can be calculated from the ratio of the area under the concentration versus time curve following oral administration (AUC_{oral}) to that following intravenous administration (AUC_{iv}) and correcting for dose (Equation 1):

(1)

$$F_{oral} = (AUC_{oral}/AUC_{iv}) \times Dose_{iv}/Dose_{oral}$$

When drug-eliminating organs are arranged in series, such as the small intestine and liver (Figure 1), F_{oral} may also be viewed as the product of the fractions of the dose that escape metabolism by each organ (Equation 2) 110J:

(2)

$$F_{oral} = F_{abs} \times F_G \times F_L$$

where, F_{abs} is the fraction of the oral dose absorbed intact across the apical (luminal) membranes of enterocytes, and F_c and F_L are the fractIons of the absorbed dose that escape

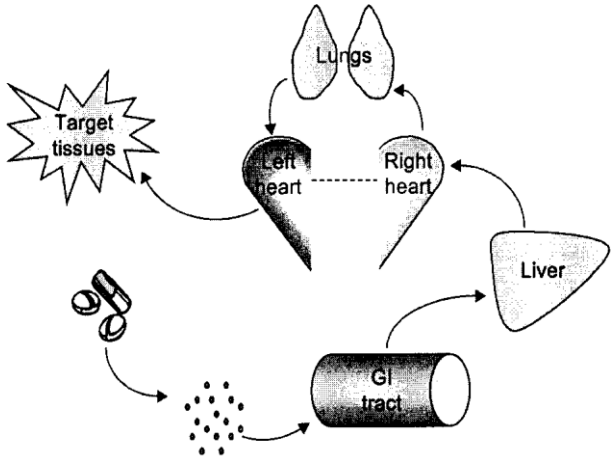

Figure 1. General anatomical path drugs follow wher taken orally.
GI: Gastrointestinal.

metabolism by the gut and liver, respectively. This simple equation demonstrates the impact of a second presystemic site of metabolism on Fora,. For example, if a drug is absorbed into enterocytes intact (F_{abs} = 100%), and has an F_G of 60% and an F_L of 40%, then an Fora, of 24% is predicted, which is roughly half the value predicted if only hepatic metabolism were considered (1 x 1 x 40% = 40%). Thus, omission of the intestinal component would lead to an overestimation of Forai, which could potentially lead to suboptimal dosing and ineffective concentrations at the site(s) of action. For drugs that have a wide therapeutic window, this can be remedied simply by increasing the dose. However, for drugs that have a narrow therapeutic window and/or exhibit a large interindividual variation in Foral, then optimization of oral drug therapy becomes more challenging. Moreover, factors that significantly influence metabolism, including other xenobiotics (either pharmaceutically or diet-derived) that induce or inhibit drug-metabolizing enzymes, thus altering FG and/or F_L, pose further challenges to effective oral drug therapy.

Equation 2 also demonstrates the potential impact of a second presystemic site of metabolism on the interindividual variation in F_{oral}. For example, if a drug has an FG that ranges 30 — 60% (twofold range), an F_L that ranges 20 — 80% (four- fold range), and the extraction efficiencies of the gut and liver vary independently (as is the case for CYP3A-mediated metabolism), then F_{oral} will range 6 — 48% (eightfold range), resulting in a 100% increase in the variation in F_{oral} (if only F_L were considered initially). Interestingly, data collected from 143 pharmacokinetic studies demonstrated a significant inverse correlation between F_{oral} and the interindividual variation in F_{oral}, as assessed by the coefficient of variation in F_{oral} [111. This relationship indicates that the greater the extent of first-pass extraction, the greater will be the variation in F_{oral}. Therefore, knowledge of the degree and variation in the expression of the major intestinal drug-metabolizing enzymes involved in drug disposition is essential, as such enzymes can represent a key determinant of the extent of first-pass elimination, as well as the interindividual variation in Fora, and the probability and magnitude of drug—drug (or drug—diet) interactions [12].

2.2 Risks of multi-drug regimens

Multi-drug regimens are common in many industrialized countries, especially among the elderly 1131, and among patients with chronic illnesses such as AIDS 1141 and cancer 1151. Because the risk of drug—drug interactions increases considerably with the number of concomitant medications, the risk of impaired treatment efficacy and/or increased drug-related toxicity will also increase [151. Reported drug—drug interactions in clinical practice frequently result from one drug impairing the elimination of another (the target). A reduced ability to eliminate the target can lead to an increase in systemic concentrations of the target, with a consequent potential for an exaggerated pharmacological or toxic biological response. The CYPs account for almost 50% of the overall elimination of commonly prescribed drugs [161. The CYP3A subfamily is believed to participate in

the metabolism of over half of therapeutic agents that undergo oxidation [16l. Taken together, inhibition of CYP3A-mediated metabolism, in the liver and/or small intestine, is a common mechanism underlying many drug—drug interactions. Moreover, the extent of inhibition by a comedicant often varies among individuals, making it challenging for the clinician to predict the magnitude and severity of the interaction, especially if the target drug has a narrow therapeutic window.

In addition to drug—drug interactions, another common concern with individuals taking multiple medications is poor adherence to the administration schedule. Associating drug administration with a regular event, such as a meal, can improve adherence [17]. A relatively overlooked consequence of this common strategy is an interaction between a drug and a dietary substance. General mechanisms underlying the effects of food on drug disposition have been well characterized and include delayed gastric emptying, solubilization of drug by food and digestive fluids, complexation of drug with food components and alterations in hepatic blood flow [18.19]. In addition, because the majority of xenobiotics that enter the body are components of the diet, it follows that some of these diet-derived xenobiotics may be capable of modulating drug metabolism in a manner similar to therapeutic agents [18,191. Therefore, inhibition of drug metabolism by dietary substances could lead to increased concentrations of the target drug and the potential for an exaggerated pharmacological response or toxicity.

3. Drug-fruit juice interactions

One of the most widely studied dietary substances shown unequivocally to inhibit drug metabolism is grapefruit juice. As detailed in subsequent sections, the consequences of such drug—grapefruit juice interactions can be clinically significant. Accordingly, a major research effort has been launched to characterize the mechanism(s) underlying, as well as the specific components that mediate, these interactions. The remainder of this review focuses on grapefruit juice and other fruit juices shown to inhibit drug metabolism *in vivo* and, perhaps more importantly, the lessons learned while delineating these relatively understudied types of drug interactions.

3.1 Grapefruit juice

More than 15 years have passed since the serendipitous observation of a pharmacokinetic interaction between the calcium-channel antagonist, felodipine, and grapefruit juice, which was used to mask the taste of ethanol during a felodipine—ethanol interaction study [201. Specifically, in patients with borderline hypertension, the combination of felodipine and a nontoxicating dose of ethanol resulted in a higher incidence of orthostatic hypotension than felodipine alone. Moreover, although plasma felodipine concentrations were not different between the two treatments, they greatly

Table 1. Controlled *in vivo* drug–diet interaction studies involving non-grapefruit juices*.

Juice	Drug tested	Species	Inhibited enteric protein	Change in mean AUC	Reference
Lime juice	Felodipine	Human	No interaction	20% ↑ (NS)	[41]
Seville orange‡	Felodipine	Human	CYP3A	76% ↑ ($p < 0.025$)	[72]
	Ciclosporin	Human	No interaction	↔ (NS)	[73]
	Saquinavir	Human	CYP3A	70% ↑ ($p = 0.04$)	[74]
	Indinavir	Human	No interaction	↔ (NS)	[77]
Pomelo	Ciclosporin	Human	CYP3A/P-gp	20% ↑ ($p = 0.0001$)	[80]
Orange (sweet)	Fexofenadine	Human	OATP	70% ↓ ($p < 0.001$)	[46]
	Celiprolol	Human	OATP(?)	83% ↓ ($p < 0.01$)	[82]
	Pravastatin	Human	P-gp/MRP2/BCRP(?)	52% ↑ ($p < 0.05$)	[84]
Cranberry	Ciclosporin	Human	No interaction	↔ (NS)	[80]
	Flurbiprofen	Human	No interaction	↔ (NS)	[94]
	Nifedipine	Rat	CYP3A	60% ↑ ($p < 0.05$)	[91]
Pomegranate	Carbamazepine	Rat	CYP3A	50% ↑ ($p < 0.01$)	[99]

*Comprehensive lists of drug–grapefruit juice interaction studies are provided in several excellent reviews [17,22,25,60,71].

‡Also known as sour orange.

BCRP: Breast cancer-resistance protein; MRP: Multi-drug resistance-associated protein; NS: Not significant; OATP: Organic anion-transporting polypeptide; P-gp: P-glycoprotein.

exceeded those observed in other pharmacokinetic studies involving the same dose of drug. After ruling out obvious potential causes (e.g., incorrect dose, drug assay problems), the investigators suspected an interaction with the grapefruit juice vehicle. A subsequent controlled clinical study confirmed grapefruit juice as the causative agent and demonstrated that this drug—diet interaction resulted from the grapefruit juice-mediated inhibition of felodipine metabolism
by CYP3A 121,221.

3.1.1 Inhibition of intestinal CYP3A

Drug—grapefruit juice interactions are distinct from the majority of drug—drug interactions in that, when the juice is consumed in usual volumes, inhibition occurs exclusively in the small intestine. Unlike other major CYPs, CYP3A is expressed in high abundance in the small intestine [4], with an expression level and associated catalytic activity in the proximal portion that are comparable to those in the liver 123,24]. Hepatic CYP3A activity appears to be unaffected by grapefruit juice, as evidenced by a lack of effect on the clearance and half-life of intravenously administered drugs [22,251, as well as on the intravenous erythromycin breath test [26]. The lack of an effect on hepatic CYP3A is believed to be due to dilution of the causative ingredient(s) in portal blood to concentrations below their effectIve inhibitory concentrations and/or to extensive binding of the causative ingredients to plasma proteins in portal blood [27].

Enteric CYP3A inhibition by grapefruit juice components include both reversible and mechanism-based modes 122,28]. In addition, the pivotal investigation by Lown *et al.* [26] demonstrated that grapefruit juice significantly reduced mean enteric CYP3A4 immunoreactive protein content (by ~ 60%), which was measured in duodenal pinch biopsies obtained, before and after juice ingestion, from nine healthy volunteers. The reduction in CYP3A4 protein was unaccompanied by a decrease in mRNA, which was believed to reflect accelerated degradation of the enzyme following mechanism-based inactivation [26]. Moreover, because inactivation of the protein is permanent, *de novo* enzyme synthesis is required to restore CYP3A4 activity. This irreversible loss of CYP3A4 protein also explains the prolonged effect of grapefruit juice (~ 3 days) [29-311. This striking observation, that a natural product was capable of destroying an endogenous protein in an irreversible fashion, further stimulated the identification of the causative ingredients responsible for the grapefruit juice effect.

3.1.2 CYP3A inhibitory ingredients

Flavonoids were originally alleged to be the CYP3A inhibitors contained in grapefruit juice, due to their high abundance in the juice and inhibitory effects *in vitro* 1321. Administration of purified forms of these compounds (naringin, quercetin) to human volunteers, however, failed to demonstrate an effect [33-35]. Numerous *in vitro* studies later indicated that furanocoumarins are the major inhibitors, acting by both reversible and irreversible mechanisms, with inhibitory constants (IC50, K) in the low micromolar range [27,28,36-40]. Bergamottin and 6',7'-dihydroxybergamottin are two abundant, and the most extensively studied, furanocoumarins, and each is generally present in grapefruit juice preparations at concentrations well above those required to effectively inhibit CYP3A activity *in vitro* [22,27,28,37,39,401. To gain further mechanistic understanding of the major ingredients responsible for the

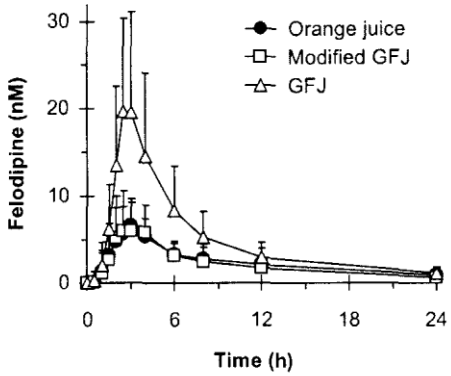

Figure 2. Mean plasma concentration versus time profile for 18 healthy volunteers orally administered a single dose of felodipine (10 mg) with 240 ml of orange juice (sweet), a modified GFJ devoid of furanocoumarins, or whole GFJ. Error bars denote standard deviations.
GFJ: Grapefruit juice.

grapefruit juice effect, the time-dependent absorption and inhibitory properties of bergamottin and 6',7'-dihydroxybergamottin were compared systematically using a human small intestine-derived cell line (Caco-2), modified to express functionally active CYP3A4 1401. A marked difference in both the rate of cell entry and onset of inhibition was observed. 6',7'-Dihydroxybergamottin rapidly diffused across the apical membranes of cells, within minutes, and effectively inhibited CYP3A4 activity, whereas the more lipophilic bergamottin had a much slower rate of entry and a delayed onset of inhibition. These results, which could not be predicted from subcellular tissue fractions (e.g., microsomes) or recombinant enzyme, indicated that enteric CYP3A4 is maximally inhibited by 6', 7'-dihydroxybergamottin before bergamottin has the opportunity to act, suggesting that 6',7'-dihydroxybergamottin represents a major CYP3A inhibitor when grapefruit juice is consumed with a rapidly absorbed substrate (e.g., midazolam, felodipine). Indeed, lime juice, which contains bergamottin at a concentration exceeding that in grapefruit juice, but no 6',7'-dihydroxybergamottin, had a minimal and insignificant effect (~ 20% increase) on average felodipine AUC in healthy volunteers (Table 1) [41J. In contrast, a grapefruit juice fraction enriched in 6',7'-dihydroxybergamottin, but with negligible bergamottin, had a substantial effect on average felodipine AUC (90% increase) [42]. Most recently, the carbonated beverage, Fresca®, which contains a high concentration of bergamottin, but no 6',7'-dihydroxybergamottin, had no effect on ciclosporin systemic exposure [431, further indicating that bergamottin is not the sole ingredient responsible for the grapefruit juice effect, even with a slowly absorbed CYP3A substrate. Furanocoumarin dimers, some of which are more potent CYP3A inhibitors than 6',7'-dihydroxybergamottin [39,44[, may represent additional major contributors to the grapefruit juice effect. Whether these compounds act as efficiently as 6', 7'-dihydroxybergamottin remains to be evaluated.

To determine whether furanocoumarins, in aggregate, account for drug—grapefruit interactions *in vivo*, the effects of a novel, food-grade grapefruit juice that was devoid of furanocoumarins (~ 99%) were tested on the pharmacokinetics of felodipine in 18 healthy volunteers [45]. As anticipated, relative to regular (sweet) orange juice (control), which does not contain furanocoumarins, whole grapefruit juice significantly increased median

felodipine AUC and C_{max} by two- to threefold, while having no effect on terminal half-life. In contrast, the modified grapefruit juice had no demonstrable effect on any pharmacokinetic outcome, with a mean felodipine concentration—time profile that was superimposable with that of orange juice (Figure 2). This study demonstrated, for the first time, that furanocoumarins, in aggregate (and potentially other unidentified bioactive compounds that were removed during preparation of the modified juice), mediate the interaction between grapefruit juice and the model CYP3A substrate felodipine *in vivo.* These substances are also likely responsible, at least in part, for interactions between grapefruit juice and other drugs that undergo extensive enteric CYP3A-mediated first-pass metabolism (e.g., several HIV protease inhibitors, immunosuppressants, calcium-channel antagonists and 3-hydroxy-3-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitors).

3.1.3 Inhibition of intestinal transporters

Although the aforementioned clinical study established furanocoumarins as mediators of the interaction between felodipine and grapefruit juice, such may not be the case for all CYP3A substrates. Unlike felodipine, several CYP3A substrates are also substrates for the efflux transporter P-glycoprotein (P-gp), which is located on the apical membrane of multiple cell types, including enterocytes. Accordingly, enteric P-gp functions to extrude its substrates back into the intestinal lumen. Taken together, inhibition of enteric P-gp activity would be expected to enhance drug systemic exposure. Dual CYP3A/P-gp substrates include some immunosuppressants (e.g., ciclosporin, tacrolimus, sirolimus), HIV protease inhibitors (e.g., saquinavir, ritonavir) and chemotherapeutic agents (e.g., paclitaxel, docetaxel). Whether or not grapefruit juice inhibits enteric P-gp activity remains controversial. For example, experiments with Caco-2 cell monolayers demonstrated that diluted grapefruit juice, or organic extracts of the juice, inhibited the bidirectional translocation of the nonmetabolized P-gp substrates, digoxin [46,47[and talinolol [481, as well as the dual CYP3A/P-gp substrates, saquinavir and vinblastine [49-5i[. However, in human volunteers, grapefruit juice had a negligible effect on the systemic exposure of digoxin [52,53[and decreased, significantly, the systemic exposure of talinolol (by 30%) [541 and two other nonmetabolized P-gp substrates, fexofenadine and celiprolol (by 60 and 85%, respectively) [46,55]. These apparent *in vitro—in vivo* inconsistencies were attributed to inhibition by grapefruit juice components of an apically located uptake transporter belonging to the organic anion-transporting polypeptide (OATP, or SLCO) family. At least one OATP (OATP-B, or OATP2B1) is expressed in enterocytes [56,57]. As such, opposite to enteric P-gp, enteric OATP functions to facilitate the absorption of its substrates, which include digoxin and fexofenadine [58,59]. Diluted grapefruit juice and some of its components, including 6',7'-dihydroxybergamottin and bergamottin, were recently reported to inhibit OATP activity in OATP-B-transfected human embryonic kidney cells [60]. Collectively, these observations support the contention that, in addition to intestinal CYP3A, the net effect of grapefruit juice may depend on the balance of intestinal uptake and efflux for certain medications [53].

3.7.4 Clinical and regulatory implications

Despite the fact that grapefruit juice acts only at the level of the intestine, the magnitude of the interaction can have clinical ramifications. For example, in case reports involving the HMG CoA reductase inhibitors, atorvastatin and simvastatin, both of which undergo extensive enteric CYP3A-mediated metabolism, consumption of fresh grapefruit was deduced to have caused muscle pain and weakness secondary to elevated circulating systemic concentrations of the `statin, [61,62]. Such adverse effects are indicative of statin-associated rhabdomyolysis, a potential life-threatening skeletal muscle toxicity. Indeed, controlled clinical studies have demonstrated that grapefruit juice can significantly increase the systemic exposure of these and other statins [63-66]. Additional drug—grapefruit juice interactions shown to result in an enhanced pharmacological effect include those involving the dihydropyridine calcium-channel antagonists, felodipine, nifedipine and nisoldipine (hypotension, increased heart rate), as well as the antiarrhythmic agent, amiodarone (proarrhythmia) [67]. As a consequence of these and other potential clinically important outcomes, the labeling for some drugs (e.g., amiodarone, verapamil, ciclosporin, sirolimus, simvastatin, lovastatin, felodipine) includes warnings or precautionary statements regarding grapefruit juice interaction potential [201]. Ciclosporin, sirolimus and other drugs with a narrow therapeutic window are of particular concern because the magnitude of the interaction is unpredictable. Such unpredictability is due in part to the large interindividual variation in baseline enteric CYP3A content/activity (8- to 10-fold) [26,68] and to the large variation in the composition of

the active ingredients in different brands, as well as different lots of the same brand, of grapefruit juice preparations [36,69.70]. It also follows that, for some drugs, variable enteric P-gp (~ 10-fold) [68] and OATP activity would contribute to the variation in response. Several excellent reviews provide a comprehensive summary of drugs shown to interact with grapefruit juice, along with the magnitude of the interaction and potential clinical outcome [17,22,25,60,71J.

3.2 Other furanocoumarin-containing citrus juices

The identification of furanocoumarins as major components responsible for drug—grapefruit juice interactions suggested that these compounds could be used as marker substances to identify other foods with interaction potential. Indeed, juices prepared from two citrus fruits related to the grapefruit have been shown to contain several of the same furanocoumarins as grapefruit juice: the Seville (sour) orange and the pomelo [28,72]. Accordingly, controlled clinical studies have been conducted to assess whether these juices act in a manner similar to grapefruit juice.

3.2.7 Seville orange juice

The Seville, or sour, orange is used primarily in the preparation of certain confectionaries, such as marmalades and candied fruit. It is not commercially available as a beverage due to an extremely sour taste. Nevertheless, because juice prepared from the fresh fruit has been reported to contain furanocoumarins at concentrations comparable to grapefruit juice [28,72], as proof of principle, the effects of a Seville orange juice have been tested on the pharmacokinetics of some CYP3A substrates (Table 1). For example, by randomized crossover design, 10 healthy volunteers were given felodipine with a single glass of Seville orange juice, diluted grapefruit juice (which contained equivalent total molar concentrations of bergamot- tin and 6',7'-dihydroxybergamottin), or regular (sweet) orange juice as the control juice [72]. Relative to control, both Seville orange juice and grapefruit juice significantly increased average felodipine AUC (by ~ 80 and 90%, respectively), although having no effect on terminal half-life. Thus, Seville orange juice appeared to act in a manner similar to grapefruit juice, most likely by inhibiting enteric CYP3A via furanocoumarins. Indeed, a single glass of Seville orange juice was shown to reduce average immunoreactive CYP3A4 protein content in duodenal pinch biopsies (by 40%) obtained from two individuals [73].

Most recently, a Seville orange juice was tested on the pharmacokinetics of the HIV protease inhibitor and dual CYP3A/P-gp substrate, saquinavir [74J. Because the saquinavir—grapefruit juice interaction is modest, producing a 50% increase in average AUC [75], 20 volunteers were tested to ensure detection of an interaction with Seville orange juice. As anticipated, relative to control (water), Seville orange juice significantly increased mean saquinavir AUC (by ~ 70%), with no effect on half-life. These results were inconsistent with those observed with another dual CYP3A/P-gp substrate, ciclosporin, which had no interaction with Seville orange juice (Table 1) [73]. Because grapefruit juice did have an interaction with the drug (55% increase in mean AUC), coupled with the 40% reduction in enteric CYP3A4 immunoreactive protein by Seville orange juice, the investigators concluded that enteric CYP3A is not a major contributor to the low F_{or}, of ciclosporin (~ 30%) and that Seville orange juice is devoid of P-gp inhibitors [73]. Based on observations from the saquinavir study, an alternative explanation is that the ciclosporin study was underpowered, as only seven subjects were enrolled. In addition, the Seville oranges used in the ciclosporin study could have contained furanocoumarins in aggregate concentrations much lower than those used in the saquinavir study, as only 6',7'-dihydroxybergamottin was measured in the ciclosporin study (~ 30 µg in both Seville orange and grapefruit juice), and no furanocoumarins were measured in the saquinavir study. Indeed, as with grapefruit juice preparations, natural products in general are known to vary considerably in their composition of bioactive components 1761.

The effects of a Seville orange juice have also been tested on the pharmacokinetics of another HIV protease inhibitor, indinavir [77]. Unlike saquinavir, indinavir has an appreciable Form (65%), and, hence, undergoes modest first-pass elimination. Thus, it was not surprising that Seville orange juice, and also grapefruit juice, had a negligible effect on indinavir systemic exposure. Again, although the concentration of 6',7'-dihydroxybergamottin was comparable between the two juices (40 µg), no other furanocoumarin was measured,

making it difficult to compare to the other drug interaction studies involving Seville orange juice, as well as to those involving grapefruit juice.

3.2.2 Pomelo juice

The pomelo is popular in many Asian countries and is typically consumed as the fresh fruit. Like Seville orange juice, at least one juice prepared from the pomelo has been reported to contain furanocoumarins (i.e., bergamottin and 6',7'-dihydroxybergamottin) in concentrations comparable to those in grapefruit juice [78]. One case has been reported of a potential interaction between tacrolimus and pomelo, in which a renal transplant recipient stabilized on tacrolimus exhibited an abnormally high trough concentration [79]. After questioning the patient about his recent activities, including dietary changes, and excluding other possible causes, the investigators deduced that the increased trough tacrolimus concentration was due to inhibition of intestinal CYP3A by furanocoumarins in the pomelo that the patient had consumed the evening prior to blood collection. Indeed, after abstaining from pomelo thereafter, the patient returned for another blood draw, and the trough concentration had returned to baseline. Based on this *in vivo* observation, and on *in vitro* results showing that a pomelo extract inhibited CYP3A and P-gp activity 147,78], a controlled clinical study was undertaken to assess whether pomelo juice produced an interaction with ciclosporin in 12 healthy volunteers (Table 1) [80]. As anticipated, pomelo juice increased modestly, but significantly, the mean AUC and C_{max} of ciclosporin, by 20 and *12%*, respectively. Unfortunately, as with the saquinavir—Seville orange juice interaction study, the pomelo juice was not characterized for furanocoumarin content, making it impossible to compare these results to those from other studies involving furanocoumarin-containing juices.

3.3 **Orange juice (sweet)**

Because regular (sweet) orange juice has been reported to have no interaction with some CYP3A substrates, including felodipine [211 and ciclosporin 181], some investigators have used this citrus juice as the control (rather than water) in drug—grapefruit juice interaction studies to better control for potential physiologic effects of the treatment juice, such as carbohydrate and calorie load [45]. To ascertain whether grapefruit juice and other fruit juices, including orange juice, inhibit P-gp activity *in vivo,* a healthy volunteer study was undertaken using fexofenadine [46], a P-gp substrate that undergoes negligible metabolism. Relative to water, orange juice unexpectedly and significantly decreased the mean AUC and C_{max} of fexofenadine (by ~ 70%) (Table 1), as did grapefruit juice (see Section 3.1.3). In addition, similar to an extract of grapefruit juice, an extract of orange juice was shown to inhibit OATP activity in rat and human OATP-transfected HeLa cells [46]. Because fexofenadine is a known OATP substrate, the mechanism underlying the fexofenadine—orange juice interaction was postulated to involve inhibition of OATP-mediated uptake. This mechanism could also underlie the orange juice-mediated reduction (80 — 90%) in the systemic exposure of the nonmetabolized P-gp substrate, celiprolol (Table 1) 182]. Because orange juice does not contain furanocoumarins, other components must be responsible for this `orange juice effect,. Candidate inhibitors include polymethoxylated flavones, which are abundant in sweet orange, but not grapefruit juice, and have been demonstrated to inhibit OATP-B, as well as P-gp, activity in various *in vitro* cell systems [49,51,60,83]. Unlike furanocoumarins, orange juice/polymethoxyflavones have been reported to have no inhibitory effect towards CYP3A activity in both human liver [83] and intestinal [451 microsomes.

In contrast to the aforementioned clinical studies, Koitabashi *et al.* [84] recently reported, in 14 healthy volunteers, that multiple glasses of orange juice significantly increased the systemic exposure (50% increase in mean AUC), but not half-life, of the OATP substrate, pravastatin (Table 1). Pravastatin, which undergoes minimal metabolism, is a substrate for not only OATP, but also P-gp and two other efflux transporters, multi-drug resistance-associated protein 2 (MRP2) and breast cancer-resistance protein (BCRP) [851, both of which have been detected in the apical membranes of human enterocytes. In addition to their ability to inhibit P-gp activity, orange juice/polymethoxyflavones have been shown to inhibit MRP2 activity in MRP2-transfected porcine kidney epithelial cells [51]. Whether these compounds inhibit BCRP activity remains to be determined. Studies in rats, in which orange juice also enhanced pravastatin systemic exposure, showed that changes in neither intestinal pH nor carbohydrate load altered the pharmacokinetics of pravastatin 184], thus ruling out these potential physicochemical effects of orange juice. Taken together, net inhibition of efflux, rather than

uptake, by orange juice components could explain this interaction. Clearly, further investigation is required to discern the mechanism underlying the pravastatin—orange juice interaction in humans.

Collectively, these *in vitro* and *in vivo* observations involving sweet orange juice further support the notion that, in addition to metabolism, the net effect of dietary substances on drug disposition may depend on the balance of intestinal uptake and efflux]53]. Moreover, this balance of enzymes/transporters may vary with substrate. Finally, none of the orange juice products used in any of these clinical studies was characterized for potential causative ingredients, again precluding interstudy comparisons.

3.4 Cranberry juice

Cranberry juice, a popular beverage since the 1930s, has been promoted heavily for its health benefits, including prevention of urinary tract infections (UTIs) [86,87], which account for > 11 million physician visits annually in the US [88]. Scientific evidence indicates that the prophylactic nature of cranberry juice is related to its antiadherent properties, rather than to urinary acidification [86,87,89]. *Escherichia coil,* the most common cause of UTIs, have hair-like fimbria that protrude from their surface. The fimbria produce adhesins that attach to receptors on uroepithelial cells. Compounds identified in cranberries that inhibit these adhesions include proanthocyanidins (condensed tannins), which are found in relatively high concentrations in *Vaccinum* berries (cranberries and blueberries) [90]. Finally, regular consumption of cranberry juice was effective in the prevention of UTI caused by antibiotic-resistant strains of *E. coil* [89]. Thus, cranberry juice could represent a natural alternative for women and the elderly with recurrent UTIs, who often require long-term antimicrobial therapy, which can increase the emergence of resistant strains of the causative organism(s).

A recent *in vivo* study in rats indicated that components in cranberry juice are capable of inhibiting enteric CYP3A activity (Table 1) [91]. Following oral administration of the CYP3A substrate nifedipine, cranberry juice appeared to inhibit enteric, but not hepatic, first-pass metabolism, as evidenced by a lack of an effect on drug systemic half-life [91]. Moreover, relative to control (saline), cranberry juice significantly increased the average AUC of oral nifedipine to an extent comparable to that with grapefruit juice 60%). Because species differences in drug-metabolizing enzymes are known to exist, comparable clinical studies are warranted to ascertain whether or not cranberry juice can enhance the systemic exposure of clinically relevant CYP3A substrates in humans. The single published clinical study so far involving a CYP3A substrate showed no interaction between ciclosporin and cranberry juice [80]. Although ciclosporin was a reasonable drug to test the effects of pomelo juice (described in Section 3.2.2), this drug was not ideal to test for a potential 'cranberry juice effect,, as it is not yet known whether cranberry juice modulates P-gp. Thus, it seems premature to initiate evaluation of the drug interaction potential of cranberry juice in humans using a dual CYP3A/P-gp substrate. In addition, as recognized by Aston *et al.* 1921, a much smaller quantity of cranberry juice (single glass, 240 ml) was consumed by the subjects in the clinical study compared with individuals taking the juice chronically for UTI prevention (> 2 — 3 glasses/day). Indeed, assuming an average body weight of a rat of 0.25 kg, the volume of juice given in the rat study (2 ml) [911 scales to 560 ml of juice for a 70-kg person, whIch is equivalent to 2 — 3 glasses of juice. Finally, as was the case for several previously described drug—fruit juice interaction studies, the cranberry juice used was not characterized for potential causative ingredients (e.g., major flavonoids, proanthocyanidins). Indeed, as Uesawa and Mohri [91] articulated, *'Naturally, the constituents of cranberry juice depend on the raw material and the manufacturing process. Discrepancies may therefore be due to the differences in the cranberry juice brands used by the researchers* . Although potential CYP3A inhibitory components in cranberry juice have yet to be identified, our extensive literature search, which included the use of natural products chemistry-specific search tools, such as the Dictionary of Natural Products and NAPRALERT, indicated that, of the > 150 compounds that have been identified in cranberry, none are furanocoumarins. As such, cranberry juice could contain nonfuranocoumarin-derived CYP3A inhibitors with unknown, or even known, mechanisms of action.

Several case reports (n = 8) have suggested an interaction between the anticoagulant, warfarin, and cranberry juice [92J, in which the International Normalized Ratio, an indicator of anticoagulation status, increased to supratherapeutic values; in one case, the International Normalized Ratio increased to > 50, and the patient

subsequently died from hemorrhage [93]. Warfarin, whose oral bioavailability exceeds 90%, exists as a racemic mixture, of which the more pharmacologically active (S)-enantiomer is metabolized primarily by hepatic CYP2C9. Accordingly, one proposed mechanism underlying this potentially lethal drug—diet interaction involves inhibition of CYP2C9 by components in cranberry juice. The one clinical study that used the CYP2C9 substrate, flurbiprofen, indicated no interaction with cranberry juice (Table 1) [94]. As with the ciclosporin study, the subjects were given only one glass of cranberry juice, and the juice was not characterized for potential causative ingredients. Taken together, more rigorous studies are warranted to rule out conclusively whether or not cranberry juice has drug interaction potential.

3.5 Pomegranate juice

Pomegranate juice has been touted by the media as a 'super- food, due to its antioxidant and chemoprevention properties [202] and to the scientific evidence showing its promise in a variety of conditions, including prostate cancer [95] and heart disease [96,97]. Pomegranate is rich in two types of polyphenolic compounds: anthocyanins, which give the fruit/juice its red color; and hydrolyzable tannins, which account for 92% of the antioxidant activity of the whole fruit 198]. Although this seemingly exotic juice has shown promise to have several health benefits, pomegranate juice may also interact with certain medications. For example, pomegranate juice was tested recently for its inhibitory effects towards the metabolism of the antiseizure agent and CYP3A substrate carbamazepine [99J. These investigators demonstrated this juice to be as effective as grapefruit juice in inhibiting carbamazepine metabolism, both *in vitro* using human liver microsomes, and *in vivo* in rats given the drug orally 199l. As has been demonstrated repeatedly with grapefruit juice and related citrus juices in humans, pomegranate juice appeared to inhibit only enteric CYP3A in rats, as exemplified by a lack of an effect on drug systemic half-life. Moreover, the average concentration—time profile of carbamazepine with pomegranate juice was superimposable with that of grapefruit juice. Compared with water, both juices significantly increased average AUC by 50% (Table 1). Finally, an experiment with closed *in situ* intestinal loops further indicated that pomegranate juice did not alter the absorption of carbamazepine, which is not a substrate for P-gp [100,101]. Although potential causative ingredients in pomegranate juice were not identified, these results suggest that the juice contains potent CYP3A inhibitors that could enhance the systemic exposure of clinically relevant substrates in humans. Of the > 100 compounds that have been identified in pomegranate *(Punka granatum)*, none are furanocoumarins 1102l. As is the case with cranberry juice, this observation, coupled with the fact that the pomegranate is not a citrus fruit, renders furanocoumarins unlikely to be the active CYP3A inhibitors. Thus, pomegranate juice could also contain nonfuranocoumarins with known and/or unknown mechanisms of action. Whether or not this increasingly popular juice interacts with drugs in humans remains to be determined. Again, as with all drug interaction studies involving natural products, it will be important to first characterize the juice for potential causative ingredients before testing, both *in vitro* and *in vivo*, to allow for between-study comparisons.

4. Conclusion

The small intestine can represent a major organ of first-pass drug elimination, the means of which occurs primarily via metabolism. As a consequence, because the small intestine and liver are arranged in series (Figure 1), drugs can undergo sequential, presystemic elimination before entering the general circulation. For many drugs, the extent of first-pass elimination can be large enough to reduce clinical effectiveness. Drugs with a narrow therapeutic window are especially vulnerable to this clinical problem and include several immunosuppressants and chemotherapeutic agents, many of which are substrates for the prominent drug-metabolizing enzyme, CYP3A, which is expressed in high abundance in the small intestine, in addition to the liver. Thus, inhibition of CYP3A by drugs or other xenobiotics can significantly elevate circulating drug concentrations, which can in turn lead to an enhanced pharmacological or a toxic biological response.

In addition to drugs, some diet-derived xenobiotics have been shown to inhibit CYP3A-mediated metabolism. Most notably, grapefruit juice has been demonstrated, unequivocally, to inhibit the first-pass metabolism of an array of CYP3A substrates. Drug—grapefruit juice interactions are unique in that, when the juice is consumed in usual volumes, inhibition occurs only in the small intestine; for some drugs, the consequences of the interaction can be clinically significant. Compounds that have been established as major mediators of the

grapefruit juice effect include furanocoumarins, which inhibit CYP3A activity by multiple mechanisms. Furanocoumarins have also been demonstrated to inhibit P-gp and the uptake transporter, OATP, but the collective data so far remain conflicting. Nevertheless, the identification of furanocoumarins as important causative ingredients has led to the identification of other citrus juices with interaction potential (e.g., Seville orange and pomelo juice). Sweet orange juice, which does not contain furanocoumarins, has also been shown to alter drug systemic exposure, presumably via inhibition of enteric OATP, P-gp and/or other uptake/efflux transporters. Candidate inhibitors include polymethoxyflavones, which do not inhibit CYP3A. Collectively, the effect of citrus juices on drug disposition is complex, involving multiple mechanisms and multiple causative ingredients.

Drug juice interactions may not be restricted to citrus fruits. For example, cranberry juice and pomegranate juice have been shown to inhibit enteric CYP3A-mediated metabolism *in vivo* in rats. Although the one published clinical study so far involving cranberry juice and a CYP3A (and P-gp) substrate, ciclosporin, showed no interaction, additional studies are required to conclusively rule out whether cranberry juice has drug interaction potential. Likewise, further research is required to determine whether pomegranate juice has drug interaction potential in humans.

Although the identification of specific dietary substances capable of altering drug disposition has been stimulated by the discovery of the grapefruit juice effect, progress in this crucial area of pharmacotherapy continues to remain sluggish. Identification of components responsible for drug—diet interactions is especially challenging. Unlike regulated drug products, of which the contents and potential interacting components are well understood, this is generally not the case for nonregulated products, particularly dietary substances. Taken together, the authors contend that a multidisciplinary, translational research approach, one that involves the collective expertise of drug metabolism scientists and natural products chemists, is required to fully delineate these relatively unexplored types of drug interactions. Such a multidisciplinary approach should enable, prospectively, the identification of clinically important drug—diet interactions. Indeed, as has been recommended for drug—herbal interactions [76l, journal editors have the opportunity to be especially influential in requiring a causative ingredient/pharmacokinetic outcome relationship as a requirement for publication, regardless of whether or not the clinical outcome is positive or negative.

5. Expert opinion

Interindividual variation in response to therapeutic agents is an ongoing problem in clinical practice. Genetic, pathophysiologic and environmental factors all contribute to this variation in response, which is in part due to the large interindividual variation in the body,s ability to rid itself of xenobiotics via metabolism and/or excretion. Of these factors, those derived from the environment perhaps have the greatest iníluence on drug elimination processes in the intestine, as the majority of these factors, especially drugs and dietary components, enter the body by the oral route and are subsequently absorbed by the enterocytes, which express a sundry of drug-metabolizing enzymes and transporters that determine, at least in part, the extent of drug systemic exposure. Thus, the likelihood of interactions between drugs and dietary components capable of modulating these enteric enzymes/transporters occurs wIth every meal.

The potential for interactions between drugs and dietary components is much greater and perhaps more challenging to predict than that between drugs, as interindividual variation in dietary habits is substantial. To investigate suspected drug—diet interactions appropriately, a more rigorous approach is required. Simply buying a natural product (juice, food, herb) off the shelf and testing it, both *in vitro* and *in vivo,* without considering the chemical makeup of that substance, provides no basis for comparison between studies, which in turn creates confusion in the literature. As a consequence, one is left without a firm recommendation regarding whether a specific food should be avoided while taking certain medications.

In addition to the drug—fruit juice interactions highlighted in this review, numerous other potential drug—diet interactions are projected to emerge, especially with the growing movement toward natural product remedies, often as an adjuvant to conventional medicine that is rarely disclosed to the physician or pharmacist [1031. As a

consequence, public health suffers because there will be an ever-increasing need to identify the causative ingredients contained in the natural product, as well as to discern the mechanisms underlying these potential drug—diet interactions. However, before initiating such research, it is imperative to design studies, whether *in vitro* or *in vivo,* in a manner that accounts for the variable composition of bioactive components contained in the 'natural, study materials. Even for the same species of plant, the concentrations of bioactive compounds can vary throughout the growing season 1104,1051; distinct plant parts (e.g., root versus stem versus leaf) produce and store compounds differently 1105,1061, and varying soil, water, sunlight and geographic conditions can alter the relative concentration of a plant,s chemical constituents 11071. Despite the fact that these variables are well known to anyone who drinks wine, harvested from the same vines, bottled by the same company, but produced in two different years, food and herbal materials are too often treated in the scientific literature, erroneously, as if they are all identical. Although this may be the standard procedure when examining a potential interaction between two drugs, which are manufactured according to good manufacturing practices to ensure that every lot is identical, evaluation of a potential interaction between a drug and dietary substances (e.g., juice, food, herb), which by their very nature are not identical, requires more sophistication to ensure that the results are both reproducible within a study and, perhaps more importantly, comparable between studies. Therefore, to elucidate mechanisms underlying drug—diet interactions, it is imperative to examine the natural product in a rigorous manner that scrutinizes the metabolic consequences of its consumption in the context of its unique chemical makeup. Such information would provide a basis for between-study comparisons, and most importantly, provide critical knowledge regarding whether a specific dietary component can be taken safely with certain medications.

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