INTEGRATED APPROACHES TO IDENTIFY AND PREDICT PHARMACOKINETIC-BASED DIETARY SUBSTANCE-DRUG INTERACTIONS

Christina S. Won

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Approved by:

Robert E. Dupuis, Pharm.D.

Craig R. Lee, Pharm.D., Ph.D.

Nicholas H. Oberlies, Ph.D.

Mary F. Paine, R.Ph., Ph.D.

Gary M. Pollack, Ph.D.

Dhiren R. Thakker, Ph.D.

ABSTRACT

CHRISTINA S. WON: Integrated Approaches to Identify and Predict Pharmacokinetic-Based Dietary Substance-Drug Interactions
(Under the direction of Mary F. Paine)

The large variation in bioactive ingredient composition inherent to natural products, including dietary substances, can confound the design and interpretation of natural product-drug interaction studies. The purpose of this dissertation was to address this overlooked issue by developing a framework to evaluate pharmacokinetic-based dietary substance-drug interactions such that ultimately, firm clinical recommendations can be made. Fruit juices represent a diverse market of popular foods containing phytochemicals that can inhibit drug metabolizing enzymes and transporters in the intestine. The potential increase or decrease in systemic drug exposure could lead to adverse effects or therapeutic failure, respectively. A multi-experimental approach utilizing *in vitro* (bioactivity-guided fractionation), *in vivo* (clinical study), and *in silico* (modeling and simulation) methods was applied to the exemplar dietary substance grapefruit juice (GFJ). GFJ has been shown to inhibit oral absorption of certain drugs that require the uptake transporter family of organic anion transporting polypeptides (OATPs) located in the intestine. The inhibitory effects of GFJ and a unique food-grade GFJ devoid of two classes of candidate OATP inhibitors, furanocoumarins and polymethoxyflavones, on intestinal OATP activity were evaluated in OATP1A2- and OATP2B1-transfected cells and in healthy volunteers. Results from the *in vitro* study were predictive of the *in vivo* study, demonstrating that furanocoumarins and polymethoxyflavones do not contribute to intestinal OATP inhibition. Bioactivity-guided fractionation of GFJ using estrone 3-sulfate as a probe substrate and OATP2B1-transfected cells yielded several potent groups of

OATP2B1 inhibitors. GFJ also has been shown to inhibit the metabolism of drugs that require the cytochrome P450 3A4 (CYP3A4) enzyme in the intestine. A population-based modeling and simulation program incorporating *in vitro* and *in vivo* data from the literature evaluated two furanocoumarins, 6',7'-dihydroxybergamottin and bergamottin, as candidate marker compounds predictive of the GFJ effect on select CYP3A4 drug substrates. Results from the *in silico* study supported both furanocoumarins as potential marker compounds. These integrated approaches address the challenges of, and begin to establish best practices for, the study of dietary substance-drug interactions. Such research methods must be refined and reinforced as concomitant intake of new (and older) natural products and drugs continues to rise.

In memory of George N. Sideris

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Finally, I would like to close by sharing one of many motivational quotes which decorated my lab desk and apartment:

> "A smooth sea never made a skillful mariner, neither do uninterrupted prosperity and success qualify men for usefulness and happiness. The storms of adversity, like those of the ocean, rouse the faculties, and excite the invention, prudence, skill, and fortitude of the voyager."
>
> – English proverb

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LIST OF ABBREVIATIONS

$(AUC_m/AUC_p)_{last}$ metabolite/parent AUC_{last} ratio

+BG 6',7'-dihydroxybergamottin plus bergamottin

1'-OH MDZ 1'-hydroxymidazolam

1X single strength

2X double strength

AA African American

ACAT advanced compartmental absorption & transit

ADAM advanced dissolution, absorption & metabolism

ADME absorption, distribution, metabolism, excretion

ANOVA analysis of variance

APAP acetaminophen

Aq aqueous

$AUC_{0-\infty}$ area under the curve from zero to infinite time

AUC_{last} area under the curve from zero to the last measured concentration

B/P blood to plasma partition ratio

BCA bicinchoninic acid

BCRP breast cancer resistance protein

BG bergamottin

bid two times daily

BLQ below limit of quantification

BSP bromosulfophthalein

BuOH butanol

C concentration

$CaCl_2$ calcium chloride

CBJ cranberry juice

CI confidence interval

Cl/F apparent oral clearance

C_{last} last measured concentration

Cl_{int}, intrinsic clearance

C_{max} maximum concentration

COS-1 CV-1 (simian) origin SV40 virus

CTRC Clinical and Translational Research Center

CV coefficient of variation

CYP cytochrome P450

d day(s)

DDIs drug-drug interactions

DHB 6',7'-dihydroxybergamottin

DMEM Dulbecco's modified Eagle's medium

DMSO dimethyl sulfoxide

E maximum effect

E1S estrone 3-sulfate

EDTA ethylenediaminetetraacetic acid

EGCG epigallocatechin gallate

ELSD evaporative light scattering detector

E_o baseline effect

ER extended release

f_a fraction available to be absorbed from dosage form

FC furanocoumarin

FDA Food and Drug Administration

$f_{u,gut}$ unbound fraction in enterocytes

$f_{u,inc}$, unbound fraction in incubation

$f_{u,plasma}$ unbound fraction in plasma

g gram

GCRC General Clinical Research Center

GFJ grapefruit juice

GI gastrointestinal

h hour

HBSS Hank's balanced salt solution

HEK human embryonic kidney

HeLa cervical cancer cells

HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HPLC high pressure liquid chromatography

HRMS high resolution mass spectrometry

IC_{50} half maximal inhibitory concentration

I_{max} maximum inhibitory effect

INR International Normalized Ratio

k_a first-order absorption rate constant

K_{app} concentration of mechanism-based inhibitor associated with half maximal inactivation rate (K_I)

KCl potassium chloride

kg kilogram

KH_2PO_4 monopotassium phosphate

K_i inhibition constant

k_{inact} maximal inactivation rate constant

K_m substrate concentration at which reaction rate is half of V_{max}

L liter

l liter

Log*P* logarithm of the octanol-water partition

M men

MDCKII Madin-Darby canine kidney type II

MDR1 gene encoding for P-glycoprotein

MDZ midazolam

MES 2-(*N*-morpholino)ethanesulfonic acid hydrate

mg milligram

mGFJ modified grapefruit juice

$MgSO_4$ magnesium sulfate

min minute

ml milliliter

mL milliliter

mM millimolar

mol mole

MOM 3-methoxymorphinan

MRP multi-drug resistance-associated protein

m/z mass to charge ratio

NaCl sodium chloride

NaOH sodium hydroxide

NC not calculated

ND not detected

NM not measured

nmol/l nanomoles per liter

NMR nuclear magnetic resonance

NR not reported

NS not statistically significant

NSP not specified

OATPs organic anion transporting polypeptides

OJ orange juice

Org organic

OST α/β organic solute transporter alpha/beta

PBPK physiologically-based pharmacokinetic

PBS phosphate-buffered saline

PD pharmacodynamics

PDA photodiode array detector

P-gp P-glycoprotein

PK pharmacokinetics

pK_a acid dissociation constant

p-nitrophenylacetate PNPA

POM pomegranate juice

qd daily

rCYP, recombinant CYP

s second

SD standard deviation

SDS sodium dodecyl sulfate

SE standard error

SLCO gene encoding for organic anion transporting polypeptides

SS statistically significant

SULTs sulfotransferases

$t_{½}$ terminal half-life

tid three times daily

t_{max} time to reach maximum concentration

UPLC ultrahigh pressure liquid chromatography\

UTI urinary tract infection

UV ultraviolet

VER verapamil

V_{max} maximum rate

V_{max}, maximum rate of metabolite formation

V_{ss} volume of distribution at steady state

w week

W women

y year

γ Hill coefficient

λ_z terminal elimination rate constant

µg microgram

µg/mL micrograms per milliliter

µM micromolar

µmol/l micromoles per liter

CHAPTER 1

INTRODUCTION

PROLOGUE

The diet is an underappreciated modifiable environmental factor that influences human health status. Pharmaceutical agents have long overshadowed dietary interventions as primary modifiers of health outcomes and disease amelioration. Multi-drug regimens have become commonplace and are expected to continue and escalate, predisposing millions of people to adverse drug reactions, as well as drug-drug interactions. The public's burgeoning interest in holistic, complementary, and alternative medicine has led to the adoption of additional practices to augment the effects of their drug therapies. Certain plant-derived chemicals (*i.e.*, phytochemicals) consumed through foods and dietary supplements, including herbal preparations, have become viable therapeutic options used in conjunction with prescribed, as well as over-the-counter, medications. This growing shift in the practice of health care and maintenance may put patients at risk for additional types of drug interactions.

Globalization has enhanced the opportunities for people to be exposed to a variety of exotic phytochemicals, as well as diverse medical practices, perceived to be beneficial to health. Innovative agricultural engineering and food fortification also have led to a rise in 'superfoods' and/or 'functional foods.' Coupled with the dangers of polypharmacy, it is inevitable that these worlds will collide, with beneficial or detrimental results. Like drug-drug interactions, food-drug interactions can be associated with highly variable pharmacokinetics (PK) of the "victim" drug, which can lead to

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adverse events or therapeutic failure. Various food products have the potential to perturb the absorption, distribution, metabolism, and excretion of medications. The underlying mechanisms can be physicochemical (*e.g.*, chelation), biochemical (*e.g.*, inhibition of drug metabolizing enzymes and/or transporters), or physiologically-based (*e.g.*, altered blood flow to intestine).

Plant-derived beverages, including fruit juices, are ubiquitous and represent a diverse market of food products. Aside from purported health benefits, fruit juices contain constituents that have been shown to inhibit drug metabolizing enzymes (*e.g.*, cytochrome P450s) and transport proteins (*e.g.*, organic anion transporting polypeptides) in the intestine, leading to altered PK of the victim drug. The clinical impact of such beverage-drug interactions is discussed in the following two parts. A review of the literature demonstrates challenges in designing, conducting, and interpreting clinical studies to assess dietary substance-drug interactions. Numerous *in vitro* and *in vivo* methods, similar to those used to bring a new chemical entity to market, are available to screen for drug interaction potential. However, results can be confounded by the between-brand variability in bioactive ingredient composition. Large variations, even within the same lot of the same brand, can contribute to varying magnitudes of effect on multiple substrates both *in vitro* and *in vivo*. A disregard for the phytochemical type and content can lead to lack of replication, as well as discrepancies between in *vitro* predictions and *in vivo* observations.

An improved understanding of the causative bioactive components and PK mechanisms is needed to provide firm recommendations to both clinicians and consumers on how to manage food-drug interactions. This critical, yet frequently overlooked, issue is the motivation for this dissertation project. The overall goal of the project was to develop a framework for how to conduct robust research on dietary substance-drug interactions utilizing *in vitro*, *in vivo*, and *in silico* strategies. Grapefruit juice was used as a model dietary substance due to the abundance of information amassed over the last two decades on the drug interactions caused by this dietary substance. Although the three approaches are discussed in the context of GFJ-drug interactions, the principles and methods can be applied to the study of drug interactions caused by botanicals intended for general human

consumption. A multi-experimental approach, which included a clinical study in healthy volunteers, *in vitro* studies with transporter-expressing cells, bioactivity-guided fractionation/isolation, and PK modeling and simulation, was utilized as outlined in the following specific aims:

Aim 1: *In vitro-in vivo* approach to elucidate the molecular and cellular factors mediating dietary substance-drug interactions

Evaluate the contribution of specific grapefruit juice (GFJ) components to the inhibition of intestinal organic anion transporting polypeptide (OATP) activity and consequent oral absorption of the transporter substrate by GFJ.

Hypothesis: *A food-grade GFJ devoid of certain classes of compounds (modified GFJ) can be used as a tool to identify or eliminate the contribution of specific classes of compounds to enteric OATP inhibition and reduction of oral absorption.*

1a. Evaluate the OATP inhibitory effect of GFJ and modified GFJ extracts *in vitro* using OATP1A2- and OATP2B1-transfected cell lines and estrone 3-sulfate and fexofenadine as substrates.

1b. Compare the magnitude of effect of GFJ and modified GFJ to water on the systemic exposure to the OATP substrate fexofenadine in healthy volunteers.

Aim 2: *In vitro* approach to identify the active ingredients responsible for the targeted bioactivity of dietary substances

Identify and characterize enteric OATP inhibitors in GFJ.

Hypothesis: *A bioactivity-guided fractionation approach can be used to isolate enteric OATP inhibitors using an established stably transfected cell system and an OATP probe substrate.*

2a. Generate GFJ fractions by a series of solvent extractions and preparative high performance liquid chromatography.

2b. Identify potent OATP inhibitory fractions by evaluating the effects of generated GFJ fractions on OATP activity using Madin-Darby Canine Kidney II (MDCK II) cells stably transfected with OATP2B1 and estrone 3-sulfate as the probe substrate.

2c. Determine the IC_{50} of individual GFJ components towards OATP2B1 using MDCKII cells stably transfected with OATP2B1 and estrone 3-sulfate as the probe substrate.

Aim 3: *In silico* approach to model *in vivo* drug disposition based on *in vitro* and human-derived parameters/data

Evaluate select CYP3A4 inhibitors in GFJ as candidate marker substances predictive of the effect of GFJ on CYP3A4 substrate PK behavior.

Hypothesis: *In vitro data encompassing absorption, distribution, elimination, and physicochemical properties of substrates and GFJ inhibitors can be integrated into a PBPK model to determine the predictive nature of marker GFJ ingredients and to predict the magnitude and variability of GFJ-drug interactions in humans.*

3a. Develop a whole-body human PBPK interaction model for select CYP3A4 substrates (midazolam, felodipine) and CYP3A4 inhibitors in GFJ (6',7'-dihydroxybergamottin, bergamottin) using *in vitro* and *in vivo* data from the literature and commercially available software programs.

3b. Compare predicted/simulated data with observed data and refine the PBPK model.

PART 1: INFLUENCE OF DIETARY SUBSTANCES ON INTESTINAL DRUG METABOLISM AND TRANSPORT

OVERVIEW

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.

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.

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 as 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 1.1 highlights other key observations since the discovery of the felodipine-GFJ interaction in 1989 [54]. Table 1.1 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.

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 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%, 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) (OATPs/Oatps) [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* x *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 1.2). 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.

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]. The efficacy and cost effectiveness of GFJ-boosted cyclosporine therapy were determined in a prospective clinical study evaluating 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.

Figure 1.1. Timeline of key observations in drug-grapefruit juice (GFJ) interaction research

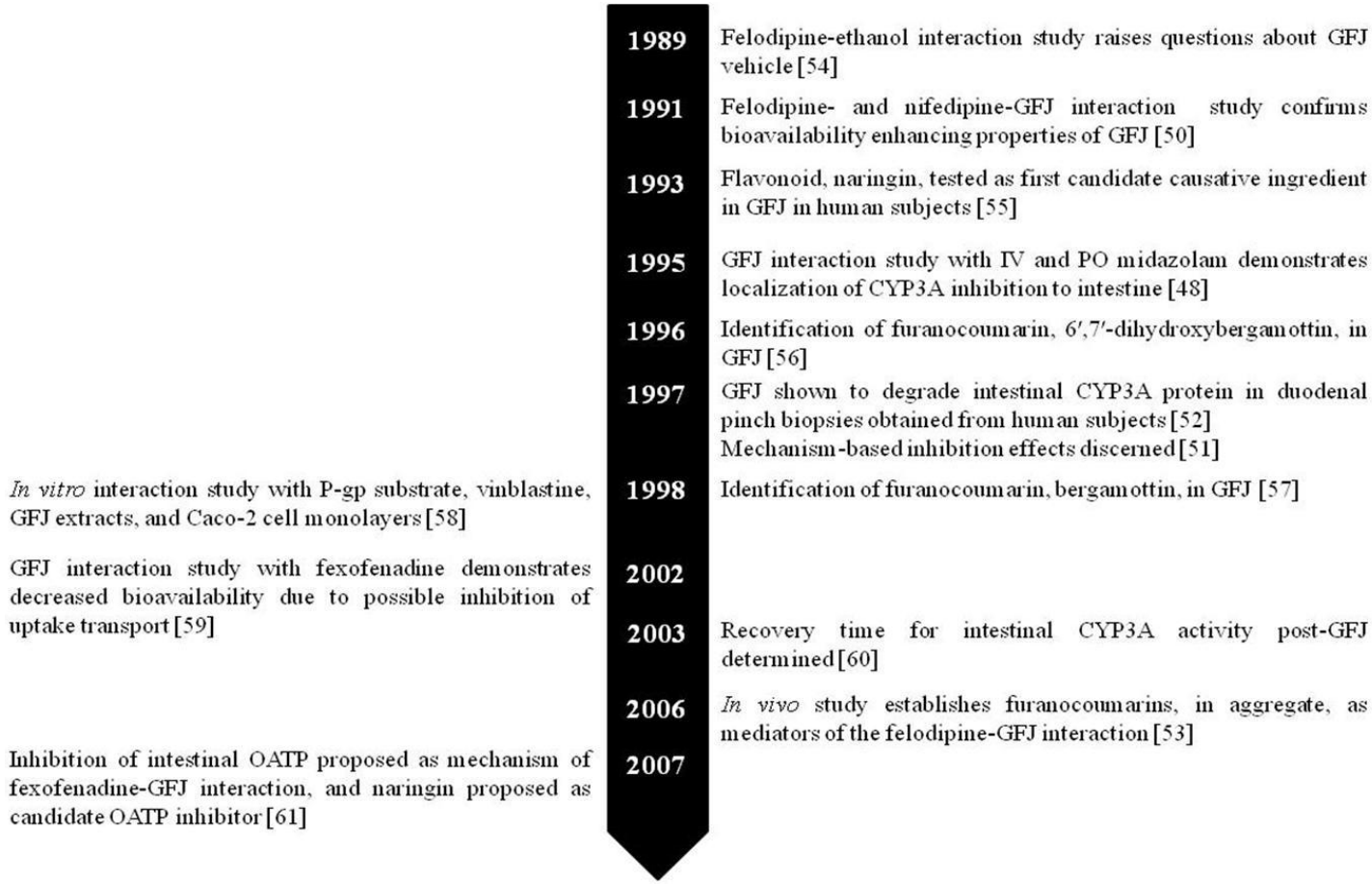

Table 1.1. Controlled clinical drug-grapefruit juice (GFJ) interaction studies reported since 2008

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 x 2d 200 mL x 2 (day 3)	↑ 30% (women) ($p = 0.01$) ↑ 11% (men) ($p = 0.27$)	62
Healthy volunteers (18)	Cyclosporine 5 mg/kg x 1	Concentrate: diluted to single strength (NSP) FC-free: single strength* 240 mL x 1	↑ 38%§ ($p < 0.01$) ↓ 1.8%§ ($p > 0.70$)	63
Healthy volunteers (19)	Dextromethorphan 10 mg (dissolved in 50 mL water) x 1	Concentrate: diluted to 0.25x, 0.5x, 1x, 2x strength (Mainfrucht GmbH & Co. KG, Gochsheim, Germany) 200 mL x 1	↓ excretion of CYP3A-dependent metabolite (MOM) by 1x and 2x strength† ($p < 0.05$)	64
Healthy men (8)	Budesonide 3 mg (as ER) x 1 (day 4)	Freshly squeezed (AstraZeneca R&D, Lund, Sweden) 200 mL tid x 3d 200 mL x 1 (day 4)	↑ 129%	65
Healthy volunteers (8)	Sertraline 75 mg x 1 (day 6)	Concentrate: diluted to single strength (Tropicana; Kirin, Tokyo, Japan) 250 mL tid x 5d 250 mL x 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 0.125x strength (Guangdong Foshan Co., China) (group B) Single strength (Tianjin Chengbao Fresh Grapefruit Juice Co., China) (group C) 250 mL bid x 1 week	↑ 22% ($p < 0.01$) ↑ 110% ($p < 0.001$)	67

Healthy men (21)	Nilotinib 400 mg x 1	Concentrate: diluted to 2x strength (Kroger Brand; The Kroger Co., Cincinnati, Ohio) 240 mL x 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 x 3d (day 25, 26, 27)	↑ 11% ($p < 0.05$)	69
Healthy volunteers (12)	Oxycodone 10 mg x 1 (day 4)	Single strength (Greippi Täysmehu; Valio Ltd., Helsinki, Finland) 200 mL tid x 5d	↑ 67% ($p < 0.001$)	70
Healthy volunteers (11)	Aliskiren 150 mg x (day 3)	Normal strength (Valio Greippitäysmehu; Valio Ltd., Helsinki, Finland) 200 mL tid x 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

Table 1.2. Clinical drug-citrus juice interaction studies since 2006 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*		63
		DHB	11.5/0.08/ND	
		BG	9.5/0.03/ND	
		Narirutin	149/104/ND	
		Naringin	440/331/ND	
		Hesperidin	8/5/ND	
		Neohesperidin	15/8/ND	
		Didymin	15/2/ND	
		Poncirin	30/9/ND	
		Nobiletin	0.56/0.01/7.66	
		Tangeretin	0.15/0.02/1.95	
	Dextromethorphan	DHB	37	64
		BG	27	
		Naringin	850	
		Naringenin	0.82	
	Sunitinib	DHB	2.7, 5.7†	69
		BG	33, 24†	
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

REFERENCES

[1] Piquette-Miller, M.; Grant, D. M. The art and science of personalized medicine. *Clin. Pharmacol. Ther.*, **2007**, *81*(3), 311-315.

[2] Robertson, G. R.; Grant, D. M.; Piquette-Miller, M. Pharmacogenetics of pharmacoecology: which route to personalized medicine? *Clin. Pharmacol. Ther.*, **2009**, *85*(4), 343-348.

[3] Koster, E. S.; Rodin, A. S.; Raaijmakers, J. A.; Maitland-van der Zee, A. H. Systems biology in pharmacogenomic research: the way to personalized prescribing? *Pharmacogenomics*, **2009**, *10*(6), 971-981.

[4] Flexner, C., Pharmacoecology: a new name for an old science. *Clin. Pharmacol. Ther.*, **2008**, *83*(3), 375-379.

[5] McLachlan, A. J.; Hilmer, S. N.; Le Couteur, D. G. Variability in response to medicines in older people: phenotypic and genotypic factors. *Clin. Pharmacol. Ther.*, **2009**, *85*(4), 431-433.

[6] Galetin, A.; Gertz, M.; Houston, J. B. Contribution of intestinal cytochrome P450-mediated metabolism to drug-drug inhibition and induction interactions. *Drug Metab. Pharmacokinet.*, **2010**, *25*(1), 28-47.

[7] Shen, D. D.; Kunze, K. L.; Thummel, K. E. Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv. Drug Deliv. Rev.*, **1997**, *27*(2-3), 99-127.

[8] Yang, J.; Jamei, M.; Yeo, K. R.; Tucker, G. T.; Rostami-Hodjegan, A. Prediction of intestinal first-pass drug metabolism. *Curr. Drug Metab.*, **2007**, *8*(7), 676-684.

[9] Galetin, A.; Gertz, M.; Houston, J. B. Potential role of intestinal first-pass metabolism in the prediction of drug-drug interactions. *Expert Opin. Drug Metab. Toxicol.*, **2008**, *4*(7), 909-922.

[10] Hisaka, A.; Ohno, Y.; Yamamoto, T.; Suzuki, H. Theoretical considerations on quantitative prediction of drug-drug interactions. *Drug Metab. Pharmacokinet.*, **2010**, *25*(1), 48-61.

[11] Galetin, A.; Hinton, L. K.; Burt, H.; Obach, R. S.; Houston, J. B. Maximal inhibition of intestinal first-pass metabolism as a pragmatic indicator of intestinal contribution to the drug-drug interactions for CYP3A4 cleared drugs. *Curr. Drug Metab.*, **2007**, *8*(7), 685-693.

[12] Fahmi, O. A.; Maurer, T. S.; Kish, M.; Cardenas, E.; Boldt, S.; Nettleton, D. A combined model for predicting CYP3A4 clinical net drug-drug interaction based on CYP3A4 inhibition, inactivation, and induction determined in vitro. *Drug Metab. Dispos.*, **2008**, *36*(8), 1698-1708.

[13] Fahmi, O. A.; Hurst, S.; Plowchalk, D.; Cook, J.; Guo, F.; Youdim, K.; Dickins, M.; Phipps, A.; Darekar, A.; Hyland, R.; Obach, R. S. Comparison of different algorithms for predicting clinical drug-drug interactions, based on the use of CYP3A4 in vitro data: predictions of compounds as precipitants of interaction. *Drug Metab. Dispos.*, **2009**, *37*(8), 1658-1666.

[14] Gertz, M.; Harrison, A.; Houston, J. B.; Galetin, A. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab. Dispos.*, **2010**, *38*(7), 1147-1158.

[15] Rowland Yeo, K.; Jamei, M.; Yang, J.; Tucker, G. T.; Rostami-Hodjegan, A. Physiologically based mechanistic modelling to predict complex drug-drug interactions involving simultaneous competitive and time-dependent enzyme inhibition by parent compound and its metabolite in both liver and gut - the effect of diltiazem on the time-course of exposure to triazolam. *Eur. J. Pharm. Sci.*, **2010**, *39*(5), 298-309.

[16] Klepser, T. B.; Doucette, W. R.; Horton, M. R.; Buys, L. M.; Ernst, M. E.; Ford, J. K.; Hoehns, J. D.; Kautzman, H. A.; Logemann, C. D.; Swegle, J. M.; Ritho, M.; Klepser, M. E. Assessment of patients' perceptions and beliefs regarding herbal therapies. *Pharmacotherapy*, **2000**, *20*(1), 83-87.

[17] Lynch, N.; Berry, D. Differences in perceived risks and benefits of herbal, over-the-counter conventional, and prescribed conventional, medicines, and the implications of this for the safe and effective use of herbal products. *Complement. Ther. Med.*, **2007**, *15*(2), 84-91.

[18] Huang, S. M.; Strong, J. M.; Zhang, L.; Reynolds, K. S.; Nallani, S.; Temple, R.; Abraham, S.; Habet, S. A.; Baweja, R. K.; Burckart, G. J.; Chung, S.; Colangelo, P.; Frucht, D.; Green, M. D.; Hepp, P.; Karnaukhova, E.; Ko, H. S.; Lee, J. I.; Marroum, P. J.; Norden, J. M.; Qiu, W.; Rahman, A.; Sobel, S.; Stifano, T.; Thummel, K.; Wei, X. X.; Yasuda, S.; Zheng, J. H.; Zhao, H.; Lesko, L. J. New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. *J. Clin. Pharmacol.*, **2008**, *48*(6), 662-670.

[19] Zhang, L.; Reynolds, K. S.; Zhao, P.; Huang, S. M. Drug interactions evaluation: an integrated part of risk assessment of therapeutics. *Toxicol. Appl. Pharmacol.*, **2010**, *243*(2), 134-145.

[20] Boullata, J. I.; Barber, J. R. A perspective on drug-nutrient interactions. In: *Handbook of drug-nutrient interactions*; Boullata, J. I., Armenti, V. T., Eds.; Humana: Totowa, 2004; pp 3-25.

[21] Santos, C. A.; Boullata, J. I. An approach to evaluating drug-nutrient interactions. *Pharmacotherapy.*, **2005**, *25*(12), 1789-1800.

[22] Genser, D. Food and drug interaction: consequences for the nutrition/health status. *Ann. Nutr. Metab.*, **2008**, *52*(Suppl 1), 29-32.

[23] Chan, L. N. Drug-nutrient interaction in clinical nutrition. *Curr. Opin. Clin. Nutr. Metab. Care*, **2002**, *5*(3), 327-332.

[24] Farkas, D.; Greenblatt, D. J. Influence of fruit juices on drug disposition: discrepancies between in vitro and clinical studies. *Expert Opin. Drug Metab. Toxicol.*, **2008**, *4*(4), 381-393.

[25] Au Yeung, S. C.; Ensom, M. H. Phenytoin and enteral feedings: does evidence support an interaction? *Ann. Pharmacother*., **2000**, *34*, 896-905.

[26] Polk, R. E. Drug-drug interactions with ciprofloxacin and other fluoroquinolones. *Am. J. Med.*, **1989**, *87*(5A), 76S-81S.

[27] Neuvonen, P. J. Interactions with the absorption of tetracyclines. *Drugs*, **1976**, *11*(1), 45-54.

[28] Singh, B. N. Effects of food on clinical pharmacokinetics. *Clin. Pharmacokinet.*, **1999**, *37*(3), 213-255.

[29] Schmidt, L. E.; Dalhoff, K. Food-drug interactions. *Drugs*, **2002**, *62*(10), 1481-1502.

[30] Fleisher, D.; Li, C.; Zhou, Y.; Pao, L. H.; Karim, A. Drug, meal and formulation interactions influencing drug absorption after oral administration. Clinical implications. *Clin. Pharmacokinet.*, **1999**, *36*(3), 233-254.

[31] Rohde, L. E.; de Assis, M. C.; Rabelo, E. R. Dietary vitamin K intake and anticoagulation in elderly patients. *Curr. Opin. Clin. Nutr. Metab. Care*, **2007**, *10*(1), 1-5.

[32] Booth, S. L.; Centurelli, M. A. Vitamin K: a practical guide to the dietary management of patients on warfarin. *Nutr. Rev.*, **1999**, *57*(9 Pt 1), 288-296.

[33] *Handbook of Drug-Nutrient Interactions*, Boullata, J. I., Armenti, V. T., Eds.; Humana: New Jersey, 2004.

[34] *Nutrition in Pharmacy Practice*, Wolinsky, I., Williams, L., Eds.; American Pharmaceutical Association: Washington, DC, 2002.

[35] Stargrove, M. B.; Treasure, J.; McKee, D. L. *Herb, Nutrient, and Drug Interactions: Clinical Implications and Therapeutic Strategies*; Mosby Elsevier: Missouri, 2008.

[36] *Handbook of Food-Drug Interactions*, McCabe, B. J., Frankel, E. H., Wolfe, J. J., Eds.; CRC Press: Florida, 2003.

[37] Espin, J. C.; Garcia-Conesa, M. T.; Tomas-Barberan, F. A. Nutraceuticals: facts and fiction. *Phytochemistry*, **2007**, *68*(22-24), 2986-3008.

[38] Qato, D. M.; Alexander, G. C.; Conti, R. M.; Johnson, M.; Schumm, P.; Lindau, S. T. Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. *J. A. M. A.*, **2008**, *300*(24), 2867-2878.

[39] Nahin, R. L.; Pecha, M.; Welmerink, D. B.; Sink, K.; DeKosky, S. T.; Fitzpatrick, A. L. Concomitant use of prescription drugs and dietary supplements in ambulatory elderly people. *J. Am. Geriatr. Soc.*, **2009**, *57*(7), 1197-1205.

[40] Loya, A. M.; Gonzalez-Stuart, A.; Rivera, J. O. Prevalence of polypharmacy, polyherbacy, nutritional supplement use and potential product interactions among older adults living on the United States-Mexico border: a descriptive, questionnaire-based study. *Drugs Aging*, **2009**, *26*(5), 423-436.

[41] Dresser, G. K.; Bailey, D. G. The effects of fruit juices on drug disposition: a new model for drug interactions. *Eur. J. Clin. Invest.*, **2003**, *33*(Suppl 2), 10-16.

[42] Lin, J. H.; Lu, A. Y. Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu. Rev. Pharmacol. Toxicol.*, **2001**, *41*, 535-567.

[43] Paine, M. F.; Hart, H. L.; Ludington, S. S.; Haining, R. L.; Rettie, A. E.; Zeldin, D. C. The human intestinal cytochrome P450 "pie". *Drug Metab. Dispos.*, **2006**, *34*(5), 880-886.

[44] Gibbs, M. A.; Hosea, N. A. Factors affecting the clinical development of cytochrome p450 3A substrates. *Clin. Pharmacokinet.*, **2003**, *42*(11), 969-984.

[45] Kim, H.; Yoon, Y. J.; Shon, J. H.; Cha, I. J.; Shin, J. G.; Liu, K. H. Inhibitory effects of fruit juices on CYP3A activity. *Drug Metab Dispos.*, **2006**, *34*(4), 521-523.

[46] Gertz, M.; Davis, J. D.; Harrison, A.; Houston, J. B.; Galetin, A. Grapefruit juice-drug interaction studies as a method to assess the extent of intestinal availability: utility and limitations. *Curr. Drug Metab.*, **2008**, *9*(8), 785-795.

[47] Huang, S. M.; Lesko, L. J. Drug-drug, drug-dietary supplement, and drug-citrus fruit and other food interactions: what have we learned? *J. Clin. Pharmacol.*, **2004**, *44*(6), 559-569.

[48] Kupferschmidt, H. H.; Ha, H. R.; Ziegler, W. H.; Meier, P. J.; Krahenbuhl, S. Interaction between grapefruit juice and midazolam in humans. *Clin. Pharmacol. Ther.*, **1995**, *58*(1), 20-28.

[49] Saito, M.; Hirata-Koizumi, M.; Matsumoto, M.; Urano, T.; Hasegawa, R. Undesirable effects of citrus juice on the pharmacokinetics of drugs: focus on recent studies. *Drug Saf.*, **2005**, *28*(8), 677-694.

[50] Bailey, D. G.; Spence, J. D.; Munoz, C.; Arnold, J. M. Interaction of citrus juices with felodipine and nifedipine. *Lancet*, **1991**, *337*(8736), 268-269.

[51] Schmiedlin-Ren, P.; Edwards, D. J.; Fitzsimmons, M. E.; He, K.; Lown, K. S.; Woster, P. M.; Rahman, A.; Thummel, K. E.; Fisher, J. M.; Hollenberg, P. F.; Watkins, P. B. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. *Drug Metab. Dispos.*, **1997**, *25*(11), 1228-1233.

[52] Lown, K. S.; Bailey, D. G.; Fontana, R. J.; Janardan, S. K.; Adair, C. H.; Fortlage, L. A.; Brown, M. B.; Guo, W.; Watkins, P. B. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J. Clin. Invest.*, **1997**, *99*(10), 2545-2553.

[53] Paine, M. F.; Widmer, W. W.; Hart, H. L.; Pusek, S. N.; Beavers, K. L.; Criss, A. B.; Brown, S. S.; Thomas, B. F.; Watkins, P. B. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. *Am. J. Clin. Nutr.*, **2006**, *83*(5), 1097-1105.

[54] Bailey, D. G.; Spence, J. D.; Edgar, B.; Bayliff, C. D.; Arnold, J. M. Ethanol enhances the hemodynamic effects of felodipine. *Clin. Invest. Med.*, **1989**, *12*(6), 357-362.

[55] Bailey, D. G.; Arnold, J. M.; Munoz, C.; Spence, J. D. Grapefruit juice--felodipine interaction: mechanism, predictability, and effect of naringin. *Clin. Pharmacol. Ther.*, **1993**, *53*(6), 637-642.

[56] Edwards, D. J.; Bellevue, F. H., 3rd; Woster, P. M. Identification of 6',7'-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. *Drug Metab. Dispos.* **1996**, *24*(12), 1287-1290.

[57] He, K.; Iyer, K. R.; Hayes, R. N.; Sinz, M. W.; Woolf, T. F.; Hollenberg, P. F. Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. *Chem. Res. Toxicol.*, **1998**, *11*(4), 252-259.

[58] Takanaga, H.; Ohnishi, A.; Matsuo, H.; Sawada, Y., Inhibition of vinblastine efflux mediated by P-glycoprotein by grapefruit juice components in Caco-2 cells. *Biol. Pharm. Bull.*, **1998**, *21*(10), 1062-1066.

[59] Dresser, G. K.; Bailey, D. G.; Leake, B. F.; Schwarz, U. I.; Dawson, P. A.; Freeman, D. J.; Kim, R. B. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin. Pharmacol. Ther.*, **2002**, *71*(1), 11-20.

[60] Greenblatt, D. J.; von Moltke, L. L.; Harmatz, J. S.; Chen, G.; Weemhoff, J. L.; Jen, C.; Kelley, C. J.; LeDuc, B. W.; Zinny, M. A. Time course of recovery of cytochrome p450 3A function after single doses of grapefruit juice. *Clin. Pharmacol. Ther.*, **2003,** *74*(2), 121-129.

[61] Bailey, D. G.; Dresser, G. K.; Leake, B. F.; Kim, R. B. Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. *Clin. Pharmacol. Ther.*, **2007**, *81*(4), 495-502.

[62] Gubbins, P. O.; Gurley, B. J.; Williams, D. K.; Penzak, S. R.; McConnell, S. A.; Franks, A. M.; Saccente, M. Examining sex-related differences in enteric itraconazole metabolism in healthy adults using grapefruit juice. *Eur. J. Clin. Pharmacol.*, **2008**, *64*(3), 293-301.

[63] Paine, M. F.; Widmer, W. W.; Pusek, S. N.; Beavers, K. L.; Criss, A. B.; Snyder, J.; Watkins, P. B. Further characterization of a furanocoumarin-free grapefruit juice on drug disposition: studies with cyclosporine. *Am. J. Clin. Nutr.*, **2008**, *87*(4), 863-871.

[64] Strauch, K.; Lutz, U.; Bittner, N.; Lutz, W. K. Dose-response relationship for the pharmacokinetic interaction of grapefruit juice with dextromethorphan investigated by human urinary metabolite profiles. *Food. Chem. Toxicol.*, **2009**, *47*(8), 1928-1935.

[65] Seidegard, J.; Randvall, G.; Nyberg, L.; Borga, O. Grapefruit juice interaction with oral budesonide: equal effect on immediate-release and delayed-release formulations. *Pharmazie*, **2009**, *64*(7), 461-465.

[66] Ueda, N.; Yoshimura, R.; Umene-Nakano, W.; Ikenouchi-Sugita, A.; Hori, H.; Hayashi, K.; Kodama, Y.; Nakamura, J. Grapefruit juice alters plasma sertraline levels after single ingestion of sertraline in healthy volunteers. *World J. Biol. Psychiatry*, **2009**, *10*(4 Pt 3), 832-835.

[67] Liu, C.; Shang, Y. F.; Zhang, X. F.; Zhang, X. G.; Wang, B.; Wu, Z.; Liu, X. M.; Yu, L.; Ma, F.; Lv, Y. Co-administration of grapefruit juice increases bioavailability of tacrolimus in liver transplant patients: a prospective study. *Eur. J. Clin. Pharmacol.*, **2009**, *65*(9), 881-885.

[68] Yin, O. Q.; Gallagher, N.; Li, A.; Zhou, W.; Harrell, R.; Schran, H. Effect of grapefruit juice on the pharmacokinetics of nilotinib in healthy participants. *J. Clin. Pharmacol.*, **2010**, *50*(2), 188-194.

[69] van Erp, N. P.; Baker, S. D.; Zandvliet, A. S.; Ploeger, B. A.; den Hollander, M.; Chen, Z.; den Hartigh, J.; Konig-Quartel, J. M.; Guchelaar, H. J.; Gelderblom, H. Marginal increase of sunitinib exposure by grapefruit juice. *Cancer Chemother. Pharmacol.* [Online early access]. DOI 10.1007/s00280-010-1367-0. Published Online: May 29, 2010. http://www.springerlink.com.libproxy.lib.unc.edu/content/p808760j34663431/ fulltext.pdf (accessed July 21, 2010).

[70] Nieminen, T. H.; Hagelberg, N. M.; Saari, T. I.; Neuvonen, M.; Neuvonen, P. J.; Laine, K.; Olkkola, K. T. Grapefruit juice enhances the exposure to oral oxycodone. *Basic Clin. Pharmacol. Toxicol.*, **2010**, *107*(4), 782-788.

[71] Tapaninen, T.; Neuvonen, P. J.; Niemi, M. Grapefruit juice greatly reduces the plasma concentrations of the OATP2B1 and CYP3A4 substrate aliskiren. *Clin. Pharmacol. Ther.*, **2010**, *88*(3), 339-342.

[72] Morton, J. F. *Fruits of Warm Climates*; Florida Flair Books: Florida, **1987**.

[73] Guo, L. Q.; Fukuda, K.; Ohta, T.; Yamazoe, Y. Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity. *Drug Metab. Dispos.*, **2000**, *28*(7), 766-771.

[74] Egashira, K.; Fukuda, E.; Onga, T.; Yogi, Y.; Matsuya, F.; Koyabu, N.; Ohtani, H.; Sawada, Y. Pomelo-induced increase in the blood level of tacrolimus in a renal transplant patient. *Transplantation*, **2003**, *75*(7), 1057.

[75] Grenier, J.; Fradette, C.; Morelli, G.; Merritt, G. J.; Vranderick, M.; Ducharme, M. P. Pomelo juice, but not cranberry juice, affects the pharmacokinetics of cyclosporine in humans. *Clin. Pharmacol. Ther.*, **2006**, *79*(3), 255-262.

[76] Guo, L. Q.; Chen, Q. Y.; Wang, X.; Liu, Y. X.; Chu, X. M.; Cao, X. M.; Li, J. H.; Yamazoe, Y. Different roles of pummelo furanocoumarin and cytochrome P450 3A5*3 polymorphism in the fate and action of felodipine. *Curr. Drug Metab.*, **2007**, *8*(6), 623-630.

[77] Al-Ghazawi, M. A.; Tutunji, M. S.; AbuRuz, S. M. The effects of pummelo juice on pharmacokinetics of sildenafil in healthy adult male Jordanian volunteers. *Eur. J. Clin. Pharmacol.*, **2010**, *66*(2), 159-163.

[78] Cote, J.; Caillet, S.; Doyon, G.; Sylvain, J. F.; Lacroix, M. Bioactive compounds in cranberries and their biological properties. *Crit. Rev. Food Sci. Nutr.*, **2010**, *50*(7), 666-679.

[79] Pappas, E.; Schaich, K. M. Phytochemicals of cranberries and cranberry products: characterization, potential health effects, and processing stability. *Crit. Rev. Food Sci. Nutr.*, **2009**, *49*(9), 741-781.

[80] Duthie, S. J.; Jenkinson, A. M.; Crozier, A.; Mullen, W.; Pirie, L.; Kyle, J.; Yap, L. S.; Christen, P.; Duthie, G. G. The effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers. *Eur. J. Nutr.*, **2006**, *45*(2), 113-122.

[81] Pérez-López, F. R.; Haya, J.; Chedraui P. *Vaccinium macrocarpon*: an interesting option for women with recurrent urinary tract infections and other health benefits. *J. Obstet. Gynaecol. Res.*, **2009**, *35*(4), 630-639.

[82] Uesawa, Y.; Mohri, K. Effects of cranberry juice on nifedipine pharmacokinetics in rats. *J. Pharm. Pharmacol.*, **2006**, *58*(8), 1067-1072.

[83] Grenier, J.; Fradette, C.; Morelli, G.; Merritt, G. J.; Vranderick, M.; Ducharme, M. P. Pomelo juice, but not cranberry juice, affects the pharmacokinetics of cyclosporine in humans. *Clin. Pharmacol. Ther.*, **2006**, *79*(3), 255-262.

[84] Lilja, J. J.; Backman, J. T.; Neuvonen, P. J. Effects of daily ingestion of cranberry juice on the pharmacokinetics of warfarin, tizanidine, and midazolam--probes of CYP2C9, CYP1A2, and CYP3A4. *Clin. Pharmacol. Ther.*, **2007**, *81*(6), 833-839.

[85] Ngo, N.; Yan, Z.; Graf, T. N.; Carrizosa, D. R.; Kashuba, A. D.; Dees, E. C.; Oberlies, N. H.; Paine, M. F. Identification of a cranberry juice product that inhibits enteric CYP3A-mediated first-pass metabolism in humans. *Drug Metab. Dispos.*, **2009**, *37*(3), 514-522.

[86] Kim, E.; Sy-Cordero, A.; Graf, T. N.; Brantley, S. J.; Paine, M. F.; Oberlies, N. H. Isolation and identification of intestinal CYP3A inhibitors from cranberry (*Vaccinium macrocarpon*) using human intestinal microsomes. *Planta Med.* [Online early access]. DOI: 10.1055/s-0030-1250259. Published Online: Aug 17, 2010. http://www.thieme-connect.de/ejournals/toc/plantamedica (accessed Aug 17, 2010).

[87] Mohammed Abdul, M. I.; Jiang, X.; Williams, K. M.; Day, R. O.; Roufogalis, B. D.; Liauw, W. S.; Xu, H.; McLachlan, A. J. Pharmacodynamic interaction of warfarin with cranberry but not with garlic in healthy subjects. *Br. J. Pharmacol.*, **2008**, *154*(8), 1691-700.

[88] Upton, R. In *American Herbal Pharmacopoeia* Cranberry Fruit: *Vaccinium macrocarpon* Aiton: Standards of Analysis, Quality Control, and Therapeutics; American Herbal Pharmacopoeia: California, 2002.

[89] Zikria, J.; Goldman, R.; Ansell, J. Cranberry juice and warfarin: when bad publicity trumps science. *Am. J. Med.*, **2010**, *123*(5), 384-392.

[90] Warfarin [package insert]. Princeton (NJ): Bristol-Myers Squibb Company; 2010.

[91] Tzulker, R.; Glazer, I.; Bar-Ilan, I.; Holland, D.; Aviram, M.; Amir, R. Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J. Agric. Food Chem.*, **2007**, *55*(23), 9559-9570.

[92] Hidaka, M.; Okumura, M.; Fujita, K.; Ogikubo, T.; Yamasaki, K.; Iwakiri, T.; Setoguchi, N.; Arimori, K. Effects of pomegranate juice on human cytochrome p450 3A (CYP3A) and carbamazepine pharmacokinetics in rats. *Drug Metab. Dispos.*, **2005**, *33*(5), 644-648.

[93] Farkas, D.; Oleson, L. E.; Zhao, Y.; Harmatz, J. S.; Zinny, M. A.; Court, M. H.; Greenblatt, D. J. Pomegranate juice does not impair clearance of oral or intravenous midazolam, a probe for cytochrome P450-3A activity: comparison with grapefruit juice. *J. Clin. Pharmacol.*, **2007**, *47*(3), 286-294.

[94] Yeo, C.; Shon, J.; Liu, K.; Lee, D.; Yoon, Y.; Shin, J. The effects of pomegranate juice on pharmacokinetics of simvastatin in healthy Korean subjects. *Clin. Pharmacol. Ther.*, **2006**, *79*(2), 23.

[95] Jarvis, S.; Li, C.; Bogle, R. G. Possible interaction between pomegranate juice and warfarin. *Emerg. Med. J.*, **2010**, *27*(1), 74-75.

[96] Komperda, K. E. Potential interaction between pomegranate juice and warfarin. *Pharmacotherapy*, **2009**, *29*(8), 1002-1006.

[97] Nagata, M.; Hidaka, M.; Sekiya, H.; Kawano, Y.; Yamasaki, K.; Okumura, M.; Arimori, K. Effects of pomegranate juice on human cytochrome P450 2C9 and tolbutamide pharmacokinetics in rats. *Drug Metab. Dispos.*, **2007**, *35*(2), 302-305.

[98] Sorokin, A. V.; Duncan, B.; Panetta, R.; Thompson, P. D. Rhabdomyolysis associated with pomegranate juice consumption. *Am. J. Cardiol.*, **2006**, *98*(5), 705-706.

[99] Hsieh, P. W.; Hung, C. F.; Fang, J. Y. Current prodrug design for drug discovery. *Curr. Pharm. Des.*, **2009**, *15*(19), 2236-2250.

[100] Walker, C. H.; Mackness, M. I. Esterases: problems of identification and classification. *Biochem. Pharmacol.*, **1983**, *32*(22), 3265-3269.

[101] Liederer, B. M.; Borchardt, R. T. Enzymes involved in the bioconversion of ester-based prodrugs. *J. Pharm. Sci.*, **2006**, *95*(6), 1177-1195.

[102] Patchett, A. A. The chemistry of enalapril. *Br. J. Clin. Pharmacol.*, **1984**, *18*(Suppl 2), 201S-207S.

[103] Sabra, R. Topics in clinical pharmacology: lovastatin. *Am. J. Med. Sci.*, **1988**, *296*(6), 426-428.

[104] Wang, E.; Casciano, C. N.; Clement, R. P.; Johnson, W. W. HMG-CoA reductase inhibitors (statins) characterized as direct inhibitors of P-glycoprotein. *Pharm. Res.*, **2001**, *18*(6), 800-806.

[105] Li, P.; Callery, P. S.; Gan, L. S.; Balani, S. K. Esterase inhibition attribute of grapefruit juice leading to a new drug interaction. *Drug Metab. Dispos.*, **2007**, *35*(7), 1023-1031.

[106] Li, P.; Callery, P. S.; Gan, L. S.; Balani, S. K. Esterase inhibition by grapefruit juice flavonoids leading to a new drug interaction. *Drug Metab. Dispos.*, **2007**, *35*(7), 1203-1208.

[107] Zhang, L.; Zuo, Z.; Lin, G. Intestinal and hepatic glucuronidation of flavonoids. *Mol. Pharm.*, **2007**, *4*(6), 833-845.

[108] Klaassen, C. D.; Boles, J. W. Sulfation and sulfotransferases 5: the importance of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) in the regulation of sulfation. *F. A. S. E. B. J.*, **1997**, *11*(6), 404-418.

[109] Gamage, N.; Barnett, A.; Hempel, N.; Duggleby, R. G.; Windmill, K. F.; Martin, J. L.; McManus, M. E. Human sulfotransferases and their role in chemical metabolism. *Toxicol. Sci.*, **2006**, *90*(1), 5-22.

[110] Coughtrie, M. W.; Johnston, L. E. Interactions between dietary chemicals and human sulfotransferases-molecular mechanisms and clinical significance. *Drug Metab. Dispos.*, **2001**, *29*(4 Pt 2), 522-528.

[111] Morgan, D. J. Clinical pharmacokinetics of beta-agonists. *Clin. Pharmacokinet.*, **1990**, *18*, 270-294.

[112] Nishimuta, H.; Ohtani, H.; Tsujimoto, M.; Ogura, K.; Hiratsuka, A.; Sawada, Y. Inhibitory effects of various beverages on human recombinant sulfotransferase isoforms SULT1A1 and SULT1A3. *Biopharm. Drug Dispos.*, **2007**, *28*(9), 491-500.

[113] Saruwatari, A.; Okamura, S.; Nakajima, Y.; Narukawa, Y.; Takeda, T.; Tamura, H. Pomegranate juice inhibits sulfoconjugation in Caco-2 human colon carcinoma cells. *J. Med. Food*, **2008**, *11*(4), 623-628.

[114] Huang, S. M.; Zhao, H.; Lee, J. I.; Reynolds, K.; Zhang, L.; Temple, R.; Lesko, L. J. Therapeutic protein-drug interactions and implications for drug development. *Clin. Pharmacol. Ther.*, **2010**, *87*(4), 497-503.

[115] Benet, L. Z. The drug transporter-metabolism alliance: uncovering and defining the interplay. *Mol. Pharm.*, **2009**, *6*(6), 1631-1643.

[116] Wacher, V. J.; Wu, C. Y.; Benet, L. Z. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol. Carcinog.*, **1995**, *13*(3), 129-134.

[117] Nowack, R.; Andrassy, J.; Fischereder, M.; Unger, M. Effects of dietary factors on drug transport and metabolism: the impact on dosage guidelines in transplant patients. *Clin. Pharmacol. Ther.*, **2009**, *85*(4), 439-443.

[118] Dahan, A.; Amidon, G. L. Grapefruit juice and its constituents augment colchicine intestinal absorption: potential hazardous interaction and the role of p-glycoprotein. *Pharm. Res.*, **2009**, *26*(4), 883-892.

[119] Bamburowicz-Klimkowska, M.; Zywiec, K.; Potentas, A.; Szutowski, M. Impact of the changes in P-glycoprotein activity on domperidone pharmacokinetics in rat plasma. *Pharmacol. Rep.*, **2007**, *59*(6), 752-756.

[120] Becquemont, L.; Verstuyft, C.; Kerb, R.; Brinkmann, U.; Lebot, M.; Jaillon, P.; Funck-Brentano, C. Effect of grapefruit juice on digoxin pharmacokinetics in humans. *Clin. Pharmacol. Ther.*, **2001**, *70*(4), 311-316.

[121] Schwarz, U. I.; Seemann, D.; Oertel, R.; Miehlke, S.; Kuhlisch, E.; Fromm, M. F.; Kim, R. B.; Bailey, D. G.; Kirch, W. Grapefruit juice ingestion significantly reduces talinolol bioavailability. *Clin. Pharmacol. Ther.*, **2005**, *77*(4), 291-301.

[122] Lilja, J. J.; Backman, J. T.; Laitila, J.; Luurila, H.; Neuvonen, P. J. Itraconazole increases but grapefruit juice greatly decreases plasma concentrations of celiprolol. *Clin. Pharmacol. Ther.*, **2003**, *73*(3), 192-198.

[123] Koitabashi, Y.; Kumai, T.; Matsumoto, N.; Watanabe, M.; Sekine, S.; Yanagida, Y.; Kobayashi, S. Orange juice increased the bioavailability of pravastatin, 3-hydroxy-3-methylglutaryl CoA reductase inhibitor, in rats and healthy human subjects. *Life Sci.*, **2006**, *78*(24), 2852-2859.

[124] Matsushima, S.; Maeda, K.; Kondo, C.; Hirano, M.; Sasaki, M.; Suzuki, H.; Sugiyama, Y. Identification of the hepatic efflux transporters of organic anions using double-transfected Madin-Darby canine kidney II cells expressing human organic anion-transporting polypeptide 1B1 (OATP1B1)/multidrug resistance-associated protein 2, OATP1B1/multidrug resistance 1, and OATP1B1/breast cancer resistance protein. *J. Pharmacol. Exp. Ther.*, **2005**, *314*(3), 1059-1067.

[125] Honda, Y.; Ushigome, F.; Koyabu, N.; Morimoto, S.; Shoyama, Y.; Uchiumi, T.; Kuwano, M.; Ohtani, H.; Sawada, Y. Effects of grapefruit juice and orange juice components on P-glycoprotein- and MRP2-mediated drug efflux. *Br. J. Pharmacol.*, **2004**, *143*(7), 856-864.

[126] Takanaga, H.; Ohnishi, A.; Yamada, S.; Matsuo, H.; Morimoto, S.; Shoyama, Y.; Ohtani, H.; Sawada, Y. Polymethoxylated flavones in orange juice are inhibitors of P-glycoprotein but not cytochrome P450 3A4. *J. Pharmacol. Exp. Ther.*, **2000**, *293*(1), 230-236.

[127] Kim, R. B. Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. *Eur. J. Clin. Invest.*, **2003**, *33*(Suppl 2), 1-5.

[128] Kalliokoski, A.; Niemi, M. Impact of OATP transporters on pharmacokinetics. *Br. J. Pharmacol.*, **2009**, *158*(3), 693-705.

[129] Hagenbuch, B.; Gui, C. Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica*, **2008**, *38*(7-8), 778-801.

[130] Hilgendorf, C.; Ahlin, G.; Seithel, A.; Artursson, P.; Ungell, A. L.; Karlsson, J. Expression of thirty-six drug transporter genes in human intestine, liver, kidney, and organotypic cell lines. *Drug Metab. Dispos.*, **2007**, *35*(8), 1333-1340.

[131] Banfield, C.; Gupta, S.; Marino, M.; Lim, J.; Affrime, M. Grapefruit juice reduces the oral bioavailability of fexofenadine but not desloratadine. *Clin. Pharmacokinet.*, **2002**, *41*(4), 311-318.

[132] Dresser, G. K.; Kim, R. B.; Bailey, D. G. Effect of grapefruit juice volume on the reduction of fexofenadine bioavailability: possible role of organic anion transporting polypeptides. *Clin. Pharmacol. Ther.*, **2005**, *77*(3), 170-177.

[133] Glaeser, H.; Bailey, D. G.; Dresser, G. K.; Gregor, J. C.; Schwarz, U. I.; McGrath, J. S.;

Jolicoeur, E.; Lee, W.; Leake, B. F.; Tirona, R. G.; Kim, R. B. Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin. Pharmacol. Ther.*, **2007**, *81*(3), 362-370.

[134] Satoh, H.; Yamashita, F.; Tsujimoto, M.; Murakami, H.; Koyabu, N.; Ohtani, H.; Sawada, Y. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab. Dispos.*, **2005**, *33*(4), 518-523.

[135] Allegra® [package insert]. Bridgewater (NJ): sanofi-aventis U.S. LLC; 2006.

[136] Lilja, J. J.; Raaska, K.; Neuvonen, P. J. Effects of grapefruit juice on the pharmacokinetics of acebutolol. *Br. J. Clin. Pharmacol.*, **2005**, *60*(6), 659-663.

[137] Reif, S.; Nicolson, M. C.; Bisset, D.; Reid, M.; Kloft, C.; Jaehde, U.; McLeod, H. L. Effect of grapefruit juice intake on etoposide bioavailability. *Eur. J. Clin. Pharmacol.*, **2002**, *58*(7), 491-494.

[138] Lilja, J. J.; Laitinen, K.; Neuvonen, P. J. Effects of grapefruit juice on the absorption of levothyroxine. *Br. J. Clin. Pharmacol.*, **2005**, *60*(3), 337-341.

[139] Greenblatt, D. J. Analysis of drug interactions involving fruit beverages and organic anion-transporting polypeptides. *J. Clin. Pharmacol.*, **2009**, *49*(12), 1403-1407.

[140] Bailey, D. G. Fruit juice inhibition of uptake transport: a new type of food-drug interaction. *Br. J. Clin. Pharmacol.* **2010**, *70*(5), 645-655.

[141] Shirasaka, Y.; Kuraoka, E.; Spahn-Langguth, H.; Nakanishi, T.; Langguth, P.; Tamai, I. Species difference in the effect of grapefruit juice on intestinal absorption of talinolol between human and rat. *J. Pharmacol. Exp. Ther.*, **2010**, *332*(1), 181-189.

[142] Nozawa, T.; Imai, K.; Nezu, J.; Tsuji, A.; Tamai, I., Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J Pharmacol Exp Ther* **2004,** *308* (2), 438-45.

[143] Mougey, E. B.; Feng, H.; Castro, M.; Irvin, C. G.; Lima, J. J. Absorption of montelukast is transporter mediated: a common variant of OATP2B1 is associated with reduced plasma concentrations and poor response. *Pharmacogenet. Genomics*., **2009**, *19*(2), 129-138.

[144] Yee, G. C.; Stanley, D. L.; Pessa, L. J. , Dalla Costa, T.; Beltz, S. E.; Ruiz, J.; Lowenthal, D. T. Effect of grapefruit juice on blood cyclosporin concentration. *Lancet*, 1995, *345*, 955-956.

[145] Lilja, J. J.; Raaska, K.; Neuvonen, P. J. Effects of orange juice on the pharmacokinetics of atenolol. *Eur. J. Clin. Pharmacol.*, **2005**, *61*(5-6), 337-340.

[146] Lilja, J. J.; Juntti-Patinen, L.; Neuvonen, P. J. Orange juice substantially reduces the bioavailability of the beta-adrenergic-blocking agent celiprolol. *Clin. Pharmacol. Ther.*, **2004**, *75*(3), 184-190.

[147] Neuhofel, A. L.; Wilton, J. H.; Victory, J. M.; Hejmanowsk, L. G.; Amsden, G. W. Lack of bioequivalence of ciprofloxacin when administered with calcium-fortified orange juice: a new twist on an old interaction. *J. Clin. Pharmacol.*, **2002**, *42*(4), 461-466.

[148] Wallace, A. W.; Victory, J. M.; Amsden, G. W. Lack of bioequivalence when levofloxacin and calcium-fortified orange juice are coadministered to healthy volunteers. *J. Clin. Pharmacol.*, **2003**, *43*(5), 539-544.

[149] Maeda, T.; Takahashi, K.; Ohtsu, N.; Oguma, T.; Ohnishi, T.; Atsumi, R.; Tamai, I. Identification of influx transporter for the quinolone antibacterial agent levofloxacin. *Mol. Pharm*, **2007**, *4*(1), 85-94.

[150] Kato, Y.; Miyazaki, T.; Kano, T.; Sugiura, T.; Kubo, Y.; Tsuji, A. Involvement of influx and efflux transport systems in gastrointestinal absorption of celiprolol. J. Pharm. Sci., **2009**, *98*(7), 2529-2539.

[151] Uesawa, Y.; Mohri, K. Hesperidin in orange juice reduces the absorption of celiprolol in rats. *Biopharm. Drug Dispos.*, **2008**, *29*(3), 185-188.

[152] Mandery, K.; Bujok, K.; Schmidt, I.; Keiser, M.; Siegmund, W.; Balk, B.; König, J.; Fromm, M. F.; Glaeser, H. Influence of the flavonoids apigenin, kaempferol, and quercetin on the function of organic anion transporting polypeptides 1A2 and 2B1. *Biochem. Pharmacol.*, **2010**, *80*(11), 1746-1753.

[153] Gerhauser, C. Cancer chemopreventive potential of apples, apple juice, and apple components. *Planta Med.*, **2008**, *74*(13), 1608-1624.

[154] Paine, M. F.; Oberlies, N. H. Clinical relevance of the small intestine as an organ of drug elimination: drug-fruit juice interactions. *Expert Opin. Drug Metab. Toxicol.*, **2007**, *3*(1), 67-80.

[155] De Castro, W. V.; Mertens-Talcott, S.; Rubner, A.; Butterweck, V.; Derendorf, H. Variation of flavonoids and furanocoumarins in grapefruit juices: a potential source of variability in grapefruit juice-drug interaction studies. *J. Agric. Food. Chem.*, **2006**, *54*(1), 249-255.

[156] Smillie, T. J.; Khan, I. A. A comprehensive approach to identifying and authenticating botanical products. *Clin. Pharmacol. Ther.*, **2010**, *87*(2), 175-186.

[157] Fenneteau, F.; Poulin, P.; Nekka, F. Physiologically based predictions of the impact of inhibition of intestinal and hepatic metabolism on human pharmacokinetics of CYP3A substrates. *J. Pharm. Sci.*, **2010**, *99*(1), 486-514.

[158] Johnson, W. W. Cytochrome P450 inactivation by pharmaceuticals and phytochemicals: therapeutic relevance. *Drug Metab. Rev.*, **2008**, *40*(1), 101-147.

[159] Iwanaga, K.; Hayashi, M.; Hamahata, Y.; Miyazaki, M.; Shibano, M.; Taniguchi, M.; Baba, K.; Kakemi, M. Furanocoumarin derivatives in Kampo extract medicines inhibit cytochrome P450 3A4 and P-glycoprotein. *Drug Metab. Dispos.*, **2008**, *38*(8), 1286-1294.

[160] Mertens-Talcott, S. U.; Zadezensky, I.; De Castro, W. V.; Derendorf, H.; Butterweck, V. Grapefruit-drug interactions: can interactions with drugs be avoided? *J. Clin. Pharmacol.*, **2006**, *46*(12), 1390-1416.

[161] http://forum.opiophile.org (accessed June 20, 2010).

[162] http://www.drugs-forum.com/forum/forumdisplay.php?f=33 (accessed June 20, 2010).

[163] Smith, N. F.; Baker, S. D.; Gonzalez, F. J.; Harris, J. W.; Figg, W. D.; Sparreboom, A. Modulation of erlotinib pharmacokinetics in mice by a novel cytochrome P450 3A4 inhibitor, BAS 100. *Br. J. Cancer.*, **2008**, *98*(10), 1630-1632.

[164] Cohen, E. E. W.; Hartford, C.; Maitland, M. L.; Fox-Kay, K.; Vogel, P.; Wu, K.; Ratain, M. J. *Grapefruit juice significantly increases bioavailability of weekly rapamycin (sirolimus) - evidence of safety and activity in a phase 1 study*, 100th Annual Meeting of the American Association for Cancer Research, Denver, CO, Apr 18-22 2009; AACR: Philadelphia, **2009**; LB-135.

[165] Ratain, M. J.; Cohen, E. E. The value meal: how to save $1,700 per month or more on lapatinib. *J. Clin. Oncol.*, **2007**, *25*(23), 3397-3398.

[166] Ioannides-Demos, L. L.; Christophidis, N.; Ryan, P.; Angelis, P.; Liolios, L.; McLean, A. J. Dosing implications of a clinical interaction between grapefruit juice and cyclosporine and metabolite concentrations in patients with autoimmune diseases. *J. Rheumatol.*, **1997**, *24*(1), 49-54.

[167] Rapamycin With Grapefruit Juice for Advanced Malignancies. http://clinicaltrials.gov/ct2/show/NCT00375245 (accessed June 21, 2010).

[168] McMains, V. Grapefruit Juice Shows Promise in Some Cancer Treatments. *Chicago Tribune* [Online] Aug 5, 2009. http://articles.chicagotribune.com/2009-08-05/news/0908050065_1_grapefruit-juice-cancer-treatments-lymph (accessed June 21, 2010).

[169] http://www.news-medical.net/news/2009/04/20/48569.aspx (accessed June 20, 2010).

[170] Watkins, P.B. The role of cytochromes P-450 in cyclosporine metabolism. *J. Am. Acad. Dermatol.*, **1990**, *23*(6), 301-311.

[171] Ducharme, M. P.; Provenzano, R.; Dehoorne-Smith, M.; Edwards, D. J. Trough concentrations of cyclosporine in blood following administration with grapefruit juice. *Br. J. Clin. Pharmacol.*, **1993**, *36*(5), 457-459.

[172] Dahan, A.; Altman, H. Food-drug interaction: grapefruit juice augments drug bioavailability—mechanism, extent and relevance. *Eur. J. Clin. Nutr*., **2004**, *58*(1), 1-9.

[173] Liu, C.; Shang, Y. F.; Zhang, X. F.; Zhang, X. G.; Wang, B.; Wu, Z.; Liu, X. M.; Yu, L.; Ma, F.; Lv, Y. Co-administration of grapefruit juice increases bioavailability of tacrolimus in liver transplant patients: a prospective study. *Eur. J. Clin. Pharmacol.*, **2009**, *65*(9), 881-885.

[174] Tsourounis, C.; Bent, S. Why change is needed in research examining dietary supplements. *Clin. Pharmacol. Ther.*, **2010**, *87*(2), 147-149.

[175] U.S. Food and Drug Administration. Guidance for Industry: Botanical Drug Products. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070491.pdf (accessed Aug 1, 2010).

[176] Schilter, B.; Andersson, C.; Anton, R.; Constable, A.; Kleiner, J.; O'Brien, J.; Renwick, A. G.; Korver, O.; Smit, F.; Walker, R. Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. *Food Chem. Toxicol.*, **2003**, *41*(12), 1625-1649.

[177] Speijers, G.; Bottex, B.; Dusemund, B.; Lugasi, A.; Tóth, J.; Amberg-Müller, J.; Galli, C. L.; Silano, V.; Rietjens, I. M. Safety assessment of botanicals and botanical preparations used as ingredients in food supplements: testing an European Food Safety Authority-tiered approach. *Mol. Nutr. Food Res.*, **2010**, *54*(2), 175-185.

[178] Dentali, S. J. Successful botanical research requires botanical expertise. *Clin. Pharmacol. Ther.*, **2010**, *87*(2), 149-151.

PART 2: MECHANISMS UNDERLYING FOOD-DRUG INTERACTIONS: INHIBITION OF INTESTINAL METABOLISM AND TRANSPORT

Overview

Food-drug interaction studies are critical to evaluate appropriate dosing, timing, and formulation of new drug candidates. These interactions often reflect prandial-associated changes in the extent and/or rate of systemic drug exposure. Physiologic and physicochemical mechanisms underlying food effects on drug disposition are well-characterized. However, biochemical mechanisms involving drug metabolizing enzymes and transport proteins remain underexplored. Several plant-derived beverages have been shown to modulate enzymes and transporters in the intestine, leading to altered pharmacokinetic (PK) and potentially negative pharmacodynamic (PD) outcomes. Commonly consumed fruit juices, teas, and alcoholic drinks contain phytochemicals that inhibit intestinal cytochrome P450 and phase II conjugation enzymes, as well as uptake and efflux transport proteins. Whereas myriad phytochemicals have been shown to inhibit these processes *in vitro*, translation to the clinic has been deemed insignificant or undetermined. An overlooked prerequisite for elucidating food effects on drug PK is thorough knowledge of causative bioactive ingredients. Substantial variability in bioactive ingredient composition and activity of a given dietary substance poses a challenge in conducting robust food-drug interaction studies. This confounding factor can be addressed by identifying and characterizing specific components, which could be used as marker compounds to improve clinical trial design and quantitatively predict food effects. Interpretation and integration of data from *in vitro*, *in vivo*, and *in silico* studies require collaborative expertise from multiple disciplines, from botany to clinical pharmacology (*i.e.*, plant to patient). Development of more systematic methods and guidelines is needed to address the general lack of information on examining drug-dietary substance interactions prospectively.

1. Introduction

The impact of food on successful delivery of promising new drug candidates *via* the oral route poses a major challenge during drug development. The influence of dietary substances on drug disposition depends on numerous variables, ranging from physicochemical properties of the drug to postprandial changes in the gastrointestinal (GI) tract [1, 2]. Components of the diet that modulate intestinal cytochrome P450 and phase II conjugation enzymes, as well as uptake and efflux transport proteins, constitute increasingly recognized contributors to food effects on drug disposition [3]. Many dietary substances or food ingredients derived from botanical sources have been shown to inhibit these processes *in vitro*, but translation to the clinic has been inconclusive or considered irrelevant [4]. Understanding the mechanisms by which these dietary substances alter drug PK and PD outcomes is critical to assess clinical significance and management.

Prediction of PK properties of new drug candidates entering clinical trials can be an arduous, sometimes elusive, task. The added complexity of food effects increases such difficulty. Robust guidelines on the evaluation of potential dietary substance-drug interactions are lacking [5]. Clinical studies often are difficult to compare, inconclusive, and/or fail to meet strict criteria required to make definitive clinical and regulatory recommendations. Commercially available modeling and simulation software can be a valuable tool to evaluate and predict, quantitatively, potential dietary substance-drug interactions. A key contributing factor to predictive success is a thorough knowledge of the causative ingredient(s) contained in the dietary substance. Identification, characterization, and validation of specific bioactive components as marker compounds can guide appropriate clinical trial design. Such studies enable development and validation of PK-PD models describing the relationship between a given dietary substance and drug of interest. The current review provides an update on dietary substance-drug interaction research, addresses challenges and potential solutions regarding the conduct and interpretation of associated studies, and discusses *in silico* strategies for predicting food effects.

2. Food-drug interactions

2.1. Definition

A food-drug interaction is the consequence of a physical, chemical, or physiologic relationship between a drug and a product consumed as food or a nutrient present in a botanically-derived food or dietary supplement [6, 7]. Such an interaction may manifest clinically as compromised health status due to altered PK and/or PD of the drug or dietary substance. Although dietary substances are regulated as food or dietary/herbal supplements, bioactive constituents in these substances can act like "perpetrator" drugs. That is, a dietary substance can increase systemic "victim" drug exposure (AUC), increasing the risk of adverse events and toxicity, or decrease systemic victim drug exposure, leading to therapeutic failure [6]. A lack of an interaction may be due to insufficient concentration(s) of causative ingredients at the enzyme or transporter active site, metabolism of causative ingredients to inactive products, or transport of causative ingredients out of target cells (*e.g.*, enterocyte, hepatocyte). Underlying mechanisms by which food exerts such effects generally include physiologic, physicochemical, and/or biochemical processes [8]. Elucidation of these processes in relevant organ systems is essential to resolve issues related to formulation, dosing schedule, and optimal pharmacotherapeutic strategies [9-11].

2.2. Regulatory guidelines

Potential clinically significant implications of food-drug interactions are recognized by worldwide regulatory agencies, each with specific guidelines. A guidance issued by the United States Food and Drug Administration (FDA) in 2002 provided recommendations on the design and conduct of food effect and fasted/fed state studies (http://www.fda.gov/regulatoryinformation/guidances). High-calorie (~800-1000 calories) and high-fat (~50% of total caloric content) test meals represent the 'worst-case scenario' and are expected to alter maximally GI physiology and subsequent systemic drug availability. Although examination of the effects of food consumption on the PK of drugs is standard practice, the issue has become greater than "take with or without food" since a variety of specific dietary substances has been shown to alter systemic drug availability. Evaluation of the

underlying mechanism(s) can ultimately lead to firm conclusions required to make informed clinical and regulatory decisions or guidelines.

3. Underlying mechanisms of food effect on drug exposure and response

3.1. Physiologic and physicochemical mechanisms

Dietary substances can alter drug absorption, distribution, metabolism, and/or excretion (ADME) *via* physiologic and physicochemical mechanisms. Physiologic/mechanical mechanisms include delayed gastric emptying, stimulated/increased bile or splanchnic blood flow, and GI pH or flora changes. Alterations of such processes can lead to reduced absorption of some drugs (*e.g.*, penicillins, angiotensin-converting enzyme inhibitors) [12]. Physicochemical mechanisms include binding of the drug by the food. For example, enteral nutrition formulas are incompatible with the antiepileptic agent, phenytoin, which can bind to proteins and salts in enteral formulations, resulting in reduced phenytoin absorption and potentially inadequate seizure control [13]. Some tetracyclines and fluoroquinolones can bind to divalent cation-containing products (*e.g.*, calcium in dairy), resulting in reduced drug absorption [14, 15] and potential therapeutic failure. High fat meals can increase drug absorption by improving solubility, such as with some antiretroviral protease inhibitors (*e.g.*, saquinavir, atazanavir) [16, 17]. Other examples are discussed comprehensively in several sources [18-22].

3.2. Biochemical mechanisms

Biochemical mechanisms include interference with co-factor formation or function, potentiation of drug PD, and modification of drug metabolizing enzyme/transporter function by the dietary substance [23]. For example, vitamin K-rich foods interfere with co-factor function and should be consumed cautiously with the anticoagulant, warfarin, as they can disrupt vitamin K metabolism and increase risk of bleeding or clot formation [24]. Isoniazid and monoamine oxidase inhibitors, used to treat tuberculosis and depression, respectively, inhibit the breakdown of endogenous and dietary amines; a tyramine-rich diet can potentiate a hypertensive crisis [25, 26]. Foods consumed as beverages account for a very high proportion of dietary antioxidant intake [27].

Growing evidence supporting cardioprotective benefits promotes moderate consumption as part of a healthy lifestyle [28, 29]. However, certain beverages contain substances that can influence drug disposition *via* modulation of drug metabolizing enzymes and transporters in the intestine.

Several studies have assessed the effect of wine, beer, fruit juices, tea, and specific constituents therein on CYP activity *in vitro*, but clinical studies are limited. These beverages have become highly recommended supplements to routinely prescribed and over-the-counter drugs and/or as monotherapy for prevention, treatment, and maintenance of common diseases. Some ingredients identified in fruit juices, teas, and alcoholic beverages have been shown to inhibit intestinal metabolism and active apical efflux/uptake processes *in vitro* and *in vivo*. 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, specifically with respect to the intestine, are highlighted in the current review. Examples of other dietary substance-drug interactions are detailed in several sources [18-22].

4. Inhibition of intestinal biochemical processes

The clinical significance of the intestine as a barrier to drug absorption and as a site for drug-drug interactions (DDIs) is recognized widely [30]. Successful delivery of an oral drug to the target site encompasses a complex multifactorial process, requiring the identification of factors and mechanisms involved in optimal formulation design and the subsequent effects of interactions with the GI environment. A prime hindrance to drug absorption is the variety of drug metabolizing enzymes and transport proteins in the enterocyte that detoxify, bioactivate, and shuttle xenobiotics (Figure 1.2). Environmental variables such as polypharmacy and diet add to the challenge of achieving therapeutic efficacy while avoiding toxicity or treatment failure [31].

Purported health benefits of certain botanical products have led to their promotion as complements (or alternatives) to drug therapy [32]. The popular consumption of fruit juices, teas, and alcoholic drinks is attributed not only to taste and nutritive value but also to increased awareness of the pharmacologic (*e.g.*, antioxidant) effects of specific constituents [33]. However, in parallel, a

growing number of *in vitro* and *in vivo* studies have demonstrated inhibitory, potentially detrimental, effects on enzymes and transporters involved in drug disposition, particularly those in the intestine, the primary portal for drugs and dietary substances [34]. The proceeding sections describe the latest findings in beverage-drug interaction research.

4.1. Phase I metabolism

4.1.1. Cytochrome P450 3A

The cytochromes P450 (CYPs) are the predominant phase I enzymes involved in drug metabolism [35]. Of the CYPs expressed in the intestine, the CYP3A subfamily is the most abundant and has been established to influence drug disposition *in vivo* [36, 37]. CYP3A is composed largely of CYP3A4 and CYP3A5 in adults. Enteric CYP3A4 is located primarily in the villous tips of the enterocytes lining the upper and middle third of the intestine (duodenum to distal jejunum) [38]. CYP3A is responsible for the oxidative metabolism of more than half of pharmaceutical agents on the market [39]. The effects of several fruit juices on CYP3A expression and activity have been studied extensively *in vitro* and in human participants. Specific inhibitory ingredients in some fruit juices have been identified and characterized. In contrast, data on enteric CYP3A inhibition by teas and alcoholic beverages (*e.g.*, wine, beer) are less abundant and the clinical significance remains to be determined.

Grapefruit juice (GFJ). Juice prepared from grapefruit (*Citrus* x *paradisi* Macfad.) is one of the most exhaustively studied dietary substances shown to inhibit enteric metabolism of numerous CYP3A substrates [40-42]. GFJ can enhance systemic drug exposure by inhibiting CYP3A-mediated pre-systemic (first-pass) metabolism in the intestine [43]. Inhibition is localized primarily in the gut, as demonstrated by a lack of effect on the elimination half-life of orally administered substrates and on the PK of intravenously administered substrates [44]. The increase in systemic drug exposure can be sufficient to produce adverse events, such as muscle pain with some statins and severe hypotension with some calcium channel blockers [45]. Compounds known as furanocoumarins (*e.g.*, 6',7'-dihydroxybergamottin, bergamottin), in aggregate, have been established as major mediators of the

'GFJ effect' in humans [43]. Modes of intestinal CYP3A inhibition include reversible and mechanism-based [46-48], as well as degradation of the protein [49]. Research on the impact of GFJ on drug disposition and response is reported and updated frequently. Table 1.3 summarizes the design and results of GFJ-drug clinical studies since the authors' previous review published in 2010 [50].

Two unusual case reports on the effect of GFJ on CYP3A substrates administered intravenously were reported recently. One involved a 52 year-old Caucasian woman diagnosed with a locally advanced unresectable esophageal squamous cell carcinoma [57]. She began a docetaxel-containing (40 mg/m^2 biweekly) chemotherapy regimen. After the first treatment cycle, the AUC of docetaxel was higher (65%) compared to typical values. In parallel, hematologic toxicity, particularly a decrease in neutrophil count (by 71%), was observed. After interviewing the patient and reviewing her medication history, the investigators concluded that GFJ was responsible for the decrease in docetaxel clearance. She reported drinking GFJ (250 mL) daily for more than three months. Two weeks after she was advised to cease drinking GFJ, docetaxel was administered. Relative to the first cycle, docetaxel AUC was reduced by 60%, approximating the AUC target value. The other case involved an 83-year-old woman with a history of acute myocardial infarction and paroxysmal atrial fibrillation [58]. She presented to the emergency department with postprandial syncope and palpitations. Pharmacologic cardioversion was initiated by administering intravenous amiodarone (300 mg loading dose). Following administration, the electrocardiogram showed marked QT prolongation associated with ventricular arrhythmias, including an episode of torsade de pointes requiring immediate electrical cardioversion. When questioned about her eating habits, the patient reported regular GFJ consumption (≥ 1-1.5 L/day). After she was advised to cease drinking GFJ, the patient recovered and was discharged four days later. These two case reports are consistent with inhibition of hepatic CYP3A by GFJ when consumed regularly in copious atypical volumes [59].

Seville orange juice. Juice prepared from the Seville (sour or bitter) orange (*Citrus* x *aurantium* L.) has been reported to contain furanocoumarins at concentrations comparable to GFJ [60, 61]. Seville orange juice has been shown to inhibit enteric CYP3A4 *in vitro* and in healthy subjects

[60-64]. Colchicine is an oral CYP3A substrate used to prevent gout flares and relieve subsequent gout attack pain [65]. The toxicities from colchicine-CYP3A inhibitor interactions can lead to multiple-organ system failure. The effect of Seville orange juice on colchicine PK was examined in healthy volunteers [54]. A single dose of colchicine (0.6 mg) was administered after a four day regimen of undiluted Seville orange juice (240 mL given twice daily). Unexpectedly, Seville orange juice decreased mean AUC of colchicine by 20% and delayed t_{max} by one hour ($p < 0.0001$) relative to water. The reduced exposure may be explained by inhibition of uptake in the intestine (see Section 4.3); however, colchicine has not been evaluated as a substrate for any uptake transport protein. This interaction is not likely to be of clinical concern, as the Seville orange is rarely consumed raw, even as juice, because of the extremely sour taste. The greatest use of Seville oranges as food is in the form of marmalade [43], but no controlled clinical studies on the effects of Seville orange marmalade consumption on drug disposition have been reported.

Pomegranate juice. The pomegranate (*Punica granatum* L.) continues as a popular 'superfood' touted for having high antioxidant content and disease prevention properties [66]. The effect of pomegranate juice on CYP3A-mediated carbamazepine metabolism was studied in human liver microsomes and in rats; results suggested pomegranate juice inhibited intestinal, but not hepatic, CYP3A activity [67]. A clinical study involving 13 healthy men given pomegranate juice (240 mL) and a single oral dose of midazolam (6 mg) suggested lack of clinical significance [68]. A more recent study involving 16 healthy Japanese volunteers evaluated the effect of repeated pomegranate juice consumption on the CYP3A-mediated metabolism of midazolam [69]. Each subject was randomized to receive either water or a commercially available normal strength pomegranate juice (200 mL) twice daily for two weeks. On day 14, midazolam (15 µg/kg) was administered orally with pomegranate juice or water. Relative to water, pomegranate juice did not significantly alter midazolam PK ($p = 0.40$). Repeated consumption of pomegranate juice may not cause a clinically relevant interaction with midazolam. However, like previous clinical studies with pomegranate juice,

no information was provided about the composition of the test juice. Therefore, generalizations about the enteric CYP3A inhibition potential of pomegranate juice are premature.

Tomato juice. The tomato fruit (*Solanum lycopersicum* L.) is consumed in many ways – raw, cooked, and in drinks. The antioxidant potency and purported anticancer properties are attributable to the rich content of lycopene, a carotene and carotenoid pigment. A recent *in vitro* study using recombinant CYP3A4 showed that tomato juice contains one or more mechanism-based and competitive inhibitor(s) of CYP3A4 [70]. Ethyl acetate extracts of three commercially available, additive-free tomato juices (A, B, and C) and homogenized fresh tomato were evaluated as inhibitors of testosterone 6β-hydroxylase activity in recombinant CYP3A4. Relative to control (absence of extract), all four extracts at 1.5% (3.75 µL extract in 250 µL of incubation mixture) inhibited activity by ~70-85%; tomato juice C also inhibited nifedipine oxidation and midazolam 1'-hydroxylation activities by 80 and 63%, respectively. The tomato juice C extract showed irreversible dose- and time-dependent, as well as partial nicotinamide adenine dinucleotide phosphate-dependent, inhibition of testosterone 6β-hydroxylation. Lycopene was tested at a concentration corresponding to that in tomato juice C (110 µg/mL of juice) but had a modest, insignificant effect (28% inhibition). The clinical significance of CYP3A4 inhibition by tomato juice has not been determined. Interestingly, tomato juice has been studied as a 'vehicle' for administering granules of the proton pump inhibitor lansoprazole [71]. Lansoprazole is metabolized by CYP2C19 and CYP3A. A randomized, four-period crossover study involving 20 healthy volunteers compared the relative oral bioavailability of lansoprazole granules administered in two juices (orange juice and tomato juice, 180 mL each) and a soft food (strained pears, 15 mL) with that of the intact capsule (30 mg) administered with water (180 mL). No differences between treatments were observed, probably because the study was not designed to evaluate the inhibitory effect of tomato juice on CYP3A. That is, lansoprazole is not an established CYP3A probe substrate, and the tomato juice product was not characterized prior to use. The possibility of a food-drug interaction between tomato juice and appropriate CYP3A substrates warrants further examination *in vivo*.

Tea. Tea is the most widely consumed beverage in the world, second only to water [72, 73]. The processing technique for the leaves of the tea plant (*Camellia sinensis* (L.) Kuntze) dictates the level of fermentation and type of tea – white, green, black, oolong, etc. [74]. Green tea undergoes minimal oxidation during processing, ensuring high polyphenol content [75]. The predominant polyphenolic compounds are catechins, which are presumed to prevent and/or treat cancer, cardiovascular disease, and obesity [76]. In addition to conventional tea infusion, concentrated green tea extract prepared in oral capsule form is sold as a dietary supplement and has become a popular option for consumers. The majority of controlled clinical studies to date evaluating the effect of repeated green tea administration (given as extract) on CYP activity has not demonstrated clinically significant interactions [77, 78]. However, a recent case report described a ~two-fold increase in tacrolimus levels observed in a 58 year-old kidney transplant recipient who ingested green tea while receiving tacrolimus [79]. Levels decreased to within the therapeutic range after discontinuation of the tea. *In vitro* and clinical studies investigating further this potential interaction have not been published. A study in rats evaluating the effect of daily green tea consumption on 5-fluorouracil PK showed green tea (50 mg/kg for four weeks) increased the AUC of 5-fluorouracil by ~425% relative to saline [80]. Larger clinical studies are needed to determine the clinical significance of these observations.

Alcoholic beverages. Growing evidence supporting cardioprotective benefits promotes moderate alcohol consumption as part of a healthy lifestyle. Alcoholic drinks such as wine and beer can alter CYP activity *via* mechanisms that are independent of ethanol [81]. Wine and beer are rich in flavonoids and other polyphenols that have antioxidant properties [82]. ***Red wine***. A handful of clinical drug interaction studies with red wine made from the common grape (*Vitis vinifera L.*) have been reported, but results have been inconsistent or clinically insignificant [83]. The magnitude of effect of red wine on the PK of CYP3A substrates may depend on both the amount and type of red wine consumed. Differentiating the effects of ethanol and wine components also poses a challenge. The red wine components *trans*-resveratrol [84] and gallic acid [85] have been shown to inhibit

hepatic CYP3A *in vitro* in a mechanism-based and non-competitive, reversible manner, respectively. However, studies on enteric inhibition are lacking. ***Beer***. Beer contains many classes of compounds, including phenolic acids, α- and β-hop acids, and prenylflavonoids [86]. Some of these compounds have been detected in the flowers of the hops plant (*Humulus lupulus* L.*)*, which are used primarily for flavoring and preserving beer [87]. Due to the resemblance to other plant-derived antioxidants and ability to inhibit CYPs that activate carcinogens, hops constituents have been studied for their chemopreventive properties. The prenylflavonoids isoxanthohumol and 8-prenylnaringenin, as well as the prenylated chalcone xanthohumol, were weak inhibitors (at a concentration of 10 μM) of nifedipine oxidase activity in recombinant CYP3A4 (data not shown in publication) [88]. The inhibitory effect of a wide range of ales, lagers, specialty beers, ciders, and non-alcoholic lagers representative of Canadian and international markets was evaluated using recombinant CYP systems [89, 90]. Major α-hop acids (*e.g.*, cohumulone, humulone, adhumulone) and β-hop acids (*e.g.*, colupulone, lupulone, adlupulone) were measured in each product, and a wide variation in contents of alcohol and hop acids was detected. Two porter ale products (10 μL in 200 μL incubation mixture, stock concentration not specified) inhibited CYP3A4-mediated dibenzylfluorescein metabolism by up to 78%. A definitive relationship between inhibition and hops constituent levels could not be established. Further studies with individual compounds are warranted to support clinical evaluation.

4.1.2. Esterase

Esterases are essential to prodrugs (*e.g.*, enalapril, lovastatin) requiring activation *via* hydrolytic cleavage of the ester bond to form the active species [91, 92]. Major esterases include carboxylesterase, acetylcholinesterase, butyrylcholinesterase, paraoxonase, and arylesterase. Inhibition of enteric esterase activity by GFJ in rats has been shown to increase 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 [93]. The clinical significance of esterase inhibition by GFJ is under investigation.

Grapefruit juice. Clopidogrel is an oral antiplatelet prodrug that is transformed *in vivo* to both an inactive metabolite *via* esterases and an active form through a series of reactions mediated by CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4 [94]. Clopidogrel is hydrolyzed by esterases to an inactive carboxylic acid derivative that accounts for 85% of circulating metabolites in plasma [95]. An ongoing clinical study (ClinicalTrials.gov Identifier: NCT00817999) is evaluating the impact of GFJ on clopidogrel loading and maintenance doses on platelet aggregation inhibition in healthy volunteers. This randomized two-way crossover study requires subjects to receive either water or single strength GFJ (325 mL) two hours prior to the loading dose (300 mg) of clopidogrel. Subjects return six hours after the loading dose to have platelet inhibition measured. A two-week washout period allows for platelet aggregation to return to baseline. The study was projected to be completed in January 2012; results await publication.

4.2. Phase II conjugation

4.2.1. Uridine diphosphate glucuronosyltransferase

Human glucuronosyl transferases (UGTs) facilitate elimination of endogenous substrates and xenobiotics by increasing hydrophilicity *via* formation of glucuronide conjugates [96]. In general, the UGTs are bound to the endoplasmic reticulum, and substrate binding sites are exposed to the lumen [97, 98]. UGTs are divided into two families, UGT1 and UGT2, which encompass more than 20 enzymes [99]. The identification of UGTs in human small intestine has been studied using a variety of approaches at different biochemical levels, from mRNA to protein to enzymatic activity. The expression of a relatively small number of UGTs has been confirmed in multiple laboratories using the same or different approaches – UGT1A1, UGT1A3, UGT1A8, UGT1A10, and UGT2B7 [100]. In contrast, analyses of several other UGTs (*e.g.*, -1A4, -1A6, -1A7, -1A9, -2B4, -2B10, -2B15) have produced conflicting results, which require further study to be resolved. The emergence of liquid chromatography-tandem mass spectrometry methods for absolute protein quantification may fill in the knowledge gap [101]. Intestinal UGTs can act to limit the oral bioavailability of many botanically-derived products. Although clinical beverage-drug interactions mediated by intestinal

UGTs have not been reported to date, the effect of diet on UGT activity in humans is discussed, as evidence supports dietary and genetic effects on UGT activity in humans [102-104].

UGT1A1 glucuronidates bilirubin, estrogens, and several dietary carcinogens [105]. Cancer chemoprevention from dietary substances occurs partly through up-regulation of UGTs. Serum bilirubin, a marker of UGT1A1 activity, was shown previously to be lower among individuals homozygous for the *UGT1A1*28* variant alleles (7/7) when randomized to a high fruit and vegetable diet [104]. In a follow-up study, healthy men (n = 146) and women (n = 147) provided blood samples for genotyping and bilirubin measurements [106]. Multiple linear regression was used to assess relationships among UGT1A1 genotype, bilirubin concentrations, and foods known to induce UGT activity (*e.g.*, cruciferous vegetables, citrus fruits, soy foods) based on three-day eating records. A significant interaction of UGT1A1 genotype and citrus consumption among women was observed. Women with the 7/7 genotype who consumed ≥0.5 daily servings of citrus fruit had ~30% lower serum bilirubin than those with the same genotype who consumed less. These results suggested that citrus consumption may increase UGT1A1 activity among women with the 7/7 genotype, potentially improving clearance of certain carcinogens and influencing cancer susceptibility.

Chemoprevention by isothiocyanates from cruciferous vegetables occurs partly through induction of UGTs. In a randomized, controlled, crossover feeding trial in humans (n = 70), three test cruciferous-based diets were compared to a fruit-and-vegetable-free basal diet [107]. Subjects were genotyped (**1/*1*, **1/*28*, **28/*28*), and serum bilirubin was measured to assess UGT1A1 activity. Aggregate bilirubin response to all cruciferous-containing diets was lower compared to the basal diet ($p < 0.03$ for all). For the *UGT1A1*28/*28* genotype, lower bilirubin concentrations were noted in all cruciferous-containing diets compared to baseline ($p < 0.02$ for all). These results may have implications for altering metabolism of both carcinogens and drugs through dietary intervention, particularly among *UGT1A1*28/*28* individuals.

Acetaminophen (APAP) glucuronidation is believed to occur mainly by UGT1A. Evidence suggests that UGT2B15 also may be important. A feeding trial was conducted to assess UGT1A6 and

UGT2B15 polymorphisms and acetaminophen conjugation in response to a randomized, controlled diet of selected fruits and vegetables known to induce UGTs (*e.g.*, cruciferous, soy, and citrus) [108]. Subjects were genotyped for *UGT1A1*28*, *UGT1A6*2*, and *UGT2B15*2*. Healthy adults (n = 66) received APAP (1 g) on days 7 and 14 of each two-week feeding period. Saliva and urine were collected over 12 hours. A modest relationship between *UGT1A6*, diet, and APAP conjugation was observed. Although the effect of the *UGT2B15*2* polymorphism on APAP glucuronidation was statistically significant, differences in APAP glucuronidation between genotypes in response to diet were small. Although larger than previous controlled feeding studies designed to evaluate effects of diet on glucuronidation, the sample size was not powered to detect genotype-diet interactions.

The inhibitory effects of commonly used herbal extracts on UGT1A4, 1A6, and 1A9 activities were evaluated in human liver microsomes [109]. The green tea constituent epigallocatechin gallate (EGCG), extracted from a green tea product, inhibited UGT1A4 activity (IC_{50} = 33.8 ± 3.1 μg/mL). UGT1A4 has been detected in the intestine [110], but green tea has not been evaluated as an intestinal UGT1A4 inhibitor *in vitro* and in humans. Further studies are warranted.

4.2.2. Sulfotransferase

Sulfotransferases (SULTs) catalyze the sulfation of a multitude of xenobiotics, hormones (*i.e.*, thyroid, estrogens), and neurotransmitters *via* conjugation with 3'-phosphoadenosine 5'-phosphosulfate [111]. Three human SULT subfamilies have been identified and detected in liver, brain, intestine, lung, kidney, and other tissues [112]. SULT1As are critical protectants from xenobiotics and ingested catecholamine precursors [113]. Molecules such as tyrosine and dopamine are sulfated preferentially by SULT1A3. Grapefruit and orange juices, as well as green tea, 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 [114-116]. SULT1A inhibition prevents normal catecholamine deactivation. Like the UGTs, clinical food-drug interactions mediated by intestinal SULTs have not been reported to date, but the effect of SULT inhibitors in the diet (*e.g.*, flavonoids in citrus fruits, wine, tea, chocolate) on circulating catecholamines has been explored. A

recent analysis of 19 published human studies showed that ingestion of SULT1A inhibitors, such as coffee (including decaffeinated), tea, chocolate, bananas, and citrus fruits can elicit catecholamine increases, blood pressure changes, migraine headaches, and/or atrial fibrillation in susceptible individuals [117]. Although diet-induced SULT1A inhibition has been shown to have serious health consequences, the impact of concomitant drug intake on this complex interaction is unknown. Controlled clinical studies with appropriate SULT substrates are needed.

4.3. Transporter-mediated efflux and uptake

4.3.1. P-glycoprotein

Inhibition of efflux transporters can lead to altered systemic and local drug concentrations. Due to the location of the efflux transporter P-glycoprotein (P-gp) on the apical (lumenal) membrane of enterocytes (Figure 1.2), substrates are extruded back into the intestinal lumen, lowering systemic drug concentrations [118]. Thus, as with CYP3A, inhibition of enteric P-gp would be expected to increase systemic drug exposure. *In vitro* studies have demonstrated inhibition of P-gp activity by citrus juices, but the clinical relevance of enteric P-gp is dubious. Whether GFJ inhibits intestinal P-gp activity *in vivo* has not been fully established, largely because an ideal P-gp probe substrate has not been identified. Several drugs transported by P-gp are metabolized by CYP3A as well [119], making it difficult to determine causality when plasma levels of a dual CYP3A/P-gp substrate are elevated post-GFJ ingestion.

4.3.2. Organic anion transporting polypeptide

The attempt to establish an *in vivo* probe for P-gp activity inadvertently gave rise to a new area of food-drug interaction studies. The initial clinical study examining effects of fruit juices on enteric P-gp activity using fexofenadine as a probe substrate showed an unexpected 63% decrease in fexofenadine AUC relative to water [120]. Mean elimination half-life was unchanged. This atypical interaction was attributed to inhibition of an apically located intestinal uptake transporter. Organic anion transporting polypeptides (OATPs) are transmembrane transport proteins that facilitate uptake of a number of endogenous compounds (*e.g.*, bile acids, hormones) and drugs [121]. Of the 11 human

OATP family members, OATP1A2 and OATP2B1 have been reported to be expressed on apical membranes of enterocytes [122] (Figure 1.2). Fruit juice inhibition of intestinal uptake transport has been reviewed [123, 124], but new studies have been reported.

Grapefruit juice. Aliskiren is a direct renin inhibitor indicated for the treatment of hypertension. A clinical study of 11 healthy volunteers administered GFJ (200 mL single-strength three times daily for five days) and aliskiren (150 mg on day 3) showed that relative to water, GFJ significantly reduced mean aliskiren AUC by 61% with no change in half-life, consistent with inhibition of intestinal but not hepatic OATPs [125]. A similar study with 28 healthy subjects receiving 300 mg aliskiren and either water or grapefruit juice (300 mL) showed a mean AUC decrease of 38% by GFJ [126]. Accompanying *in vitro* studies in human embryonic kidney 293 cells expressing OATP1A2 and OATP2B1 demonstrated that aliskiren was not taken up in OATP2B1-expressing cells, indicating aliskiren is not a substrate for OATP2B1. However, uptake of [^{14}C]-aliskiren was linear in OATP1A2-expressing cells and was reduced by the citrus fruit flavonoid naringin. The IC_{50} averaged 75 μM, which is well below the reported range of concentrations in grapefruit juice (170 μM - 6.5 mM) [127-131], supporting naringin as a candidate inhibitor of intestinal OATP. The clinical impact of the GFJ-aliskiren interaction may be minimal in view of interim results of the Aliskiren Trial in Type 2 Diabetes Using Cardio-Renal Endpoints (ALTITUDE) [132]. Safety warnings recommending discontinuation of aliskiren in patients with type 2 diabetes, renal impairment, and/or cardiovascular disease have been issued due to potential risks of renal and cardiac adverse events (http://www.pharma.us.novartis.com).

Celiprolol is a cardioselective β-adrenergic receptor blocker that has been shown to interact with GFJ [133]. One of the explanations for the observed 85% decrease in mean AUC was inhibition of celiprolol absorption in the intestine by GFJ. An uptake study in *Xenopus laevis* oocytes injected with either OATP1A2 or OATP2B1 cRNA showed that celiprolol was a substrate of OATP1A2 but not OATP2B1 [134]. In contrast, the authors of a more recent clinical interaction study hypothesized that celiprolol was transported by intestinal OATP2B1 [135], partly because the intestinal expression

of OATP2B1 is higher than that of other OATP isoforms [136, 137]. Before the human study was initiated, the authors performed an *in vitro* study in OATP2B1-expressing oocytes, demonstrating that celiprolol was transported by OATP2B1. The potential importance of pharmacogenomics led the authors to evaluate the contribution of *SLCO2B1* polymorphisms to celiprolol PK and to investigate the interaction between celiprolol and GFJ. Healthy men (n = 30) ingested 200 mL of GFJ three times daily for two days. On day 3, celiprolol (100 mg) was administered with 200 mL of GFJ. Additional GFJ (200 mL) was administered at 30 minutes and 1.5 hours thereafter. The mean AUC of celiprolol was 50% lower in *SLCO2B1*3/*3* (n = 4) than in **1/*1* (n = 5) individuals but was not deemed significant ($p = 0.10$). GFJ reduced mean AUC of celiprolol by up to 86% ($p < 0.01$) compared to water, but *SLCO2B1*3* genotype-dependent differences in the PK profiles of celiprolol disappeared in the interaction phase. Although a population PK analysis showed *SLCO2B1* status to be a statistically significant predictor of celiprolol PK, a larger trial is needed to confirm the clinical impact of the *SLCO2B1*3* polymorphism.

The clinical significance of the *SLCO2B1*3* polymorphism has been investigated for the leukotriene receptor antagonist montelukast, which is prescribed to control asthma symptoms in adults [138] and children [139]. *In vitro* experiments with Madin-Darby Canine Kidney Type II cells stably expressing OATP2B1 and Caco-2 cell lines showed that montelukast was a substrate for both OATP1A2 and OATP2B1 [138]. The PK and PD of a single dose of montelukast with and without GFJ were evaluated in adolescents and young adults (15-18 years old) with asthma [139]. The *SLCO2B1*3* polymorphism was expected to be associated with reduced absorption of montelukast, and co-ingestion of GFJ was hypothesized to decrease absorption of montelukast through inhibition of OATP2B1. Study volunteers (n = 26) were given montelukast (10 mg) with 240 mL of either Gatorade® (control), 4X concentrated GFJ, normal-strength GFJ, or normal-strength orange juice. A majority of the volunteers were *SLCO2B1*3/*3* (n = 21), while the remainder was *SLCO2B1*1/*3* (n = 5). GFJ, 4X GFJ, and orange juice decreased montelukast mean AUC by 7%, 8%, and 20%, respectively, compared to control. The reduction in mean AUC with orange juice co-ingestion in

*SLCO2B1*3/*3* individuals was considered significant ($p = 0.032$). *SLCO2B1*1/*3* individuals showed a mean AUC reduction of 37% ($p = 2 \times 10^{-5}$) compared to the **1/*3* group, independent of treatment. Despite significant PK findings, neither genotype nor co-ingestion of citrus juice had an effect on montelukast PD (data not shown in publication). However, the sample size was not justified, so the study design may have lacked sufficient power to detect an interaction. The interpretation of montelukast-*SLCO2B1* studies has been questioned by the manufacturer (Merck & Co., Inc.) [140]. A series of *in vitro* OATP2B1-related transport experiments demonstrated that there was no direct evidence indicating that montelukast is a substrate of OATP2B1 or that a *SLCO2B1* polymorphism alters montelukast uptake. OATP1A2-mediated uptake of montelukast was not discussed. Based on the conflicting evidence, conclusions on the direct involvement of OATP2B1 in the absorption of montelukast are premature, and larger trials are needed to demonstrate reproducibility of the clinical observations.

Orange juice. The sweet orange (*Citrus* x *sinensis* (L.) Osbeck) is considered fairly innocuous due to the lack of CYP3A-inhibiting furanocoumarins in the fruit. However, clinical studies with fexofenadine, certain β-blockers, and fluoroquinolones have demonstrated that orange juice can reduce systemic exposure by up to 83% [120, 141-144]. Some of these interactions have been shown *in vitro* to involve inhibition of enteric OATP by orange juice [120, 134, 145]. The effect of orange juice on aliskiren PK and PD has been investigated [146]. In a randomized crossover study, 12 healthy volunteers ingested 200 mL of orange juice, apple juice, or water three times daily for five days. On day 3, they ingested a single 150 mg dose of aliskiren. Orange juice reduced aliskiren geometric mean AUC by 62% relative to water while having no effect on elimination half-life. Plasma renin activity was increased significantly ($p = 0.037$) in the juice phase, but changes in blood pressure and heart rate were not detected. The subjects were genotyped for *SLCO2B1* polymorphisms, but no differences in aliskiren PK between the groups were observed. As discussed previously, aliskiren is not a substrate of OATP2B1. The effect of orange juice on OATP1A2-mediated aliskiren

transport has not been examined *in vitro*, but naringin, an ingredient in orange juice (and other citrus fruits), has been shown to reduce aliskiren uptake [126].

Apple juice. The consumption of apples *(Malus* x *domestica* Borkh.) has been linked to reduced risk of some cancers, cardiovascular disease, asthma, and diabetes [147]. Main structural classes of apple constituents include hydroxycinnamic acids, dihydrochalcones, flavan-3-ols, flavonols, and triterpenoids. While there have been no *in vitro* and clinical studies investigating the effect of apple juice on drug metabolism, evidence exists that apple juice inhibits OATP activity *in vitro* [148] and in human volunteers [120]. The following clinical studies involving apple juice address growing attempts to elucidate the pharmacogenomic impact of drug transport proteins.

The effects of the *SLCO2B1*3* variant (c.1457C>T) and concomitant apple juice intake on fexofenadine and midazolam oral PK were evaluated in a randomized crossover study of 14 healthy volunteers [149]. Individuals were divided based on genotype – CC (n = 5), CT (n = 5), TT (n = 4). Fexofenadine (60 mg) and midazolam (5 mg) were administered with 300 mL of either water or normal-strength apple juice (reconstituted from concentrate). Additional juice was administered post-dose (150 mL every 30 minutes for 3 hours; total volume = 1.2 L). When the genotyped groups were stratified in the water phase, subjects with CT and TT alleles showed a 37% decrease ($p < 0.05$) in fexofenadine mean AUC compared to those with CC alleles. Regardless of genotype, apple juice decreased fexofenadine mean AUC by 79% ($p < 0.05$) relative to water. Neither the genotype nor the apple juice showed significant effects on the PK of midazolam, indicating that apple juice had minimal effect on CYP3A activity. OATP-mediated uptake of fexofenadine was evaluated in *Xenopus laevis* oocytes expressing OATP2B1 and was shown to be three-fold higher ($p < 0.05$) than that by water-injected oocytes, indicating that fexofenadine was a substrate of OATP2B1. Some studies have shown that fexofenadine is transported by OATP2B1 [135, 138, 150], but others have not [151, 152]. Although the clinical study results are consistent with previous findings, the impact of the OATP2B1 polymorphism is uncertain given the small sample size.

A recently reported clinical study demonstrated that apple juice, like orange juice [141], reduced plasma concentrations of the β-blocker atenolol [153]. The effect of apple juice, as well as *SLCO2B1* c.1457C>T polymorphism, on atenolol PK was determined by a three-way crossover study of 12 healthy volunteers. Individuals were divided based on genotype – CC (n = 6) and TT (n = 6). Subjects ingested atenolol (50 mg) with either water or apple juice (300 mL) in the first phase and additional apple juice in the second (900 mL) and third (300 mL) phases. Blood pressure and heart rate were measured up to 48 hours after dosing. Apple juice (1.2 L) reduced atenolol mean AUC by up to 86% ($p < 0.001$). Genotype did not affect atenolol PK. No significant differences were observed in the hemodynamic variables. The mechanism of this interaction has not been elucidated. Atenolol has been identified as a substrate of OATP1A2 in an *in vitro* study of *Xenopus laevis* oocytes [134], but the effect of apple juice (and orange juice) has not been assessed. Although no changes in PD outcomes were observed in healthy volunteers, the apple juice-atenolol interaction may be significant in cardiovascular disease patients.

Tea. Green tea is characterized by high concentrations of catechins, including EGCG, epicatechin (EC), epigallocatechin (EGC), and epicatechin gallate (ECG). The predominant catechin, EGCG, has been studied extensively for purported health benefits [154]. The effects of the four catechins on OATP1A2 and OATP2B1 activity have been evaluated *in vitro*. Both EGCG and ECG at 100 μM inhibited OATP2B1-mediated estrone-3-sulfate uptake by ~70% [155, 156], with ECG showing higher potency than EGCG (IC_{50} of 36 *vs.* 100 μM) [156]. EGCG and ECG (both at 100 μM) also inhibited OATP1A2-mediated estrone-3-sulfate uptake by ~75%, with ECG again showing higher potency than EGCG (IC_{50} of 10 *vs*. 55 μM) [156]. ECG and EGCG concentrations in brewed green tea average 450 μM and 430 μM, respectively, with maximum concentrations of each catechin in the low millimolar range (http://www.nal.usda.gov). Consumption of a cup (*e.g.*, 240-300 mL) or two of green tea would result in intestinal concentrations of ECG and EGCG within the range that inhibits OATP activity. However, the clinical significance of intestinal OATP inhibition by green tea has not been investigated.

5. Challenges in establishing clinical significance

A number of botanically-derived beverages have been shown to inhibit several intestinal CYPs and transporters *in vitro*, but many of the interactions have not translated to the clinic. These *in vitro-in vivo* discordances may be due to a lack of sufficient and/or quality data to determine a true positive interaction (Table 1.4). Although this review is focused on beverage-drug interactions and associated mechanisms, the proceeding concepts and recommendations for how to advance research strategies and standards in this field can be applied, in general, to any botanically-derived product-drug interactions.

5.1. Methods to improve research practices

Clinical dietary substance-drug interaction studies can be confounded easily by the documented variability of specific constituents (known *and* unknown) in individual foods. Some *in vitro-in vivo* discrepancies can be addressed simply by improved documentation of the botanical source material's origin(s); others may require more rigorous experimental investigation (*e.g.*, discerning additive, synergistic, or inhibitory effects of constituents). Many clinical studies in the literature are incomplete, flawed, or superficial. Strict peer review of relevant manuscripts is essential to promoting best practices. A checklist of recommended questions to consider when evaluating submitted research articles or already published literature is listed in Table 1.5. Robust, systematic methods for evaluating potential dietary substance-drug interactions are critical, as one of the ultimate goals is to establish a framework for the quantitative prediction of food-drug interactions.

5.1.1. Identification and characterization of causative ingredients

A fundamental deficiency common to most dietary substance-drug interaction studies is limited or no descriptions of the chemical composition of the food product. The concentration of putative ingredients might not be sufficient to inhibit metabolism/transport *in vivo* [4]. Since beverages are derived from single or multiple botanical sources, they are composed of complex mixtures of numerous phytochemical entities [158]. The sources and complexity of a plant's chemical constituents can contribute to batch-to-batch inconsistency, as concentrations of bioactive compounds

can be affected by ecology, cultivation and manufacturing practices, storage conditions, and other environmental factors [129]. Thus, testing a random product *in vitro* and *in vivo* without evaluating the chemical makeup provides no basis for comparison between studies. Documentation and reporting of dietary substance characterization must be improved because many clinical studies lack thorough information about the test product. For commercially available products, the brand name, manufacturer, lot number, ingredients, preparation directions, and manufacturing process should be stated at minimum [159]. For freshly prepared material, scientific name, quantity, plant part used, collection site, preparation procedures, and storage conditions should be noted. Following verification of the test material, identification of components responsible for metabolism- or transport-mediated interactions poses the next challenge.

Thorough characterization of constituents is a difficult but essential task, and advances in analytical detection technologies have improved the efficiency and sensitivity with which active constituents have been identified. The resulting 'marker' compound(s) can be used to authenticate the test product, serving as an indicator of quality and potency in terms of standardization [160]. Since administration of 'standardized' foods is not possible, it would be more realistic to quantify a particular known/suspected component, or group of components, prior to clinical use. This practice would allow some degree of between-study comparison, as terms such as 'normal,' 'regular,' 'single,' 'double' strength have little meaning. For drug metabolism/transport purposes, a marker compound need not be unique to a particular product. The identity of constituent(s) should be confirmed by *in vitro* methods that screen for potential interactions. Selection of bioassay systems and associated protocols to quantify activity should be determined by the drug(s) and relevant phytochemical species of interest [161]. Such experiments provide mechanistic information about inhibitory capacities, as well as specific enzymes and/or transporters involved. Two examples illustrating the aforementioned approach as it applies to drug metabolism and transport are discussed [162, 163].

Conflicting results from an *in vivo* rat study [164] and a clinical study [165] examining the interaction between CYP3A substrates and cranberry juice (CBJ) prompted a systematic *in vitro-in*

vivo approach to identify a CBJ product capable of inhibiting enteric CYP3A in human volunteers [166]. The effects of five CBJ products (blinded for *in vitro* experiments) were evaluated on midazolam 1'-hydroxylation activity in human intestinal microsomes. Each juice brand (ranging from concentrate to fresh pressed preparations) inhibited CYP3A activity in a concentration-dependent manner but with differing extents of inhibition, demonstrating that one brand is not representative of the broad marketplace. The most potent of these juices, CBJ 'E', was tested next in a proof-of-concept clinical study involving 16 healthy volunteers. Each volunteer was administered juice E (double-strength) or water (240 mL x 3, separated by 15 min intervals) and midazolam (5 mg) with the third glass of juice/water. Relative to water, CBJ increased the geometric mean AUC of midazolam by ~30% ($p < 0.001$) while having no effect on geometric mean terminal half-life, suggesting inhibition of intestinal CYP3A activity.

A common approach used in the natural products field to isolate therapeutically active ingredients, bioactivity-guided fractionation, was used to identify the CYP3A inhibitors present in the clinical test CBJ [162]. The juice was partitioned into hexane-, chloroform-, butanol-, and water-soluble fractions. Each fraction was evaluated as an inhibitor of midazolam 1'-hydroxylation in human intestinal microsomes. The hexane- and chloroform-soluble fractions at 50 μg/mL were the most potent, inhibiting activity by 77 and 63%, respectively, suggesting that the CYP3A inhibitors resided in these more lipophilic fractions. A series of bioactivity-guided fractionation studies with whole cranberry (*Vaccinium macrocarpon* Ait.) were initiated next to identify potential specific enteric CYP3A inhibitors in cranberry [162]. Using human intestinal microsomes and recombinant CYP3A4, three triterpenes (maslinic acid, corosolic acid, ursolic acid) were isolated. IC_{50} values ranged from 2.8 (maslinic acid) to <10 μM (ursolic acid). Results suggested that these triterpenes may have contributed to the CBJ-midazolam interaction observed clinically. The overall approach substantiated that *in vitro* characterization of dietary substances is required before initiation of clinical dietary substance-drug interaction studies, one brand of a given dietary substance is not predictive of

all brands, and bioactivity-guided fractionation can be used to identify specific causative bioactive ingredients.

A bioassay-directed isolation approach was applied to the subtropical *shrub* aratiku (*Rollinia emarginata* Schlecht) to identify OATP modulators [163]. The stem barks of the shrub have been used in combination with yerba mate (*Ilex paraguayensis* St. Hilaire) as a migraine treatment and as a relaxant. Initial screening identified aratiku extract as a modulator of OATP-mediated transport. Fractions of the extract were screened in Chinese hamster ovary cells stably transfected with OATP1B1 or OATP1B3. Potential effects on OATP1B1- and OATP1B3-mediated uptake of the two model substrates, estradiol-17β-glucuronide and estrone-3-sulfate, were evaluated. Although the *in vitro* experiments were intended for liver-specific OATPs, to the best of the authors' knowledge, the approach is the only one published to date that utilized bioactivity-guided fractionation to isolate modulators of drug uptake transporters. Ursolic acid, oleanolic acid, and 8-*trans*-*p*-coumaroyloxy-α-terpineol significantly inhibited estradiol-17β-glucuronide uptake by OATP1B1 (~70% of DMSO control) but not OATP1B3. The IC_{50} values for ursolic acid and oleanolic acid were ~15 μM and ~4 μM, respectively. Ursolic acid and oleanolic acid modestly inhibited estrone-3-sulfate uptake by both OATPs (by up to 40% of control). Quercetin 3-*O*-α-L-arabinopyranosyl(1→2) α –L-rhamnopyranoside strongly inhibited OATP1B1- and OATP1B3-mediated uptake of estradiol-17β-glucuronide (by > 95%). However, OATP1B3-mediated uptake of estrone-3-sulfate was stimulated. The thorough descriptions of the starting material, preparation procedures, analytical methods, and bioassay system protocols were strengths of the work. The overall method illustrated the efficiency of bioassay-guided isolation to identify selective transporter modulators in a botanical that has not been studied clinically. Interestingly, ursolic acid also was identified in the CBJ product mentioned previously. Such information permits assumptions about which drugs may interact with a food containing ursolic acid (*e.g.*, apple, cranberry, pear, plum, olives) [167-170].

Identification and thorough characterization of a particular dietary substance does not change immediately certain research practices. Since the establishment of furanocoumarins in GFJ as

unequivocal mediators of enteric CYP3A-based interactions in 2006 [37], only a handful (n ~ 5) of clinical studies involving CYP3A substrates have reported furanocoumarin content in the test juice. Furanocoumarins have been studied to the extent that they can be considered marker compounds. Characterization of a given GFJ in terms of furanocoumarin content could be used to predict the likelihood and magnitude of an interaction (see Section 5.1.4). Between-study comparisons also can be made. A basic, yet overlooked, solution to establishing meaningful physiological dose-response relationships for dietary substances is to characterize the product prior to use. The information gained will inform the nature and extent of follow-up *in vitro* and *in vivo* interaction studies.

5.1.2. Appropriate design of in vitro and in vivo food-drug interaction studies

The regulation (or lack thereof) of dietary substances in the United States is different from drugs. Although dietary substances are regulated as food [5], bioactive compounds in these substances can act like drugs (*i.e.*, cure, mitigate, treat, or prevent disease). Since it is unlikely that legislation will change to require more intense drug-dietary substance interaction studies, individuals in the field can take a cue from the pharmaceutical industry and adopt some or all recommendations made in the FDA's guidance on drug-drug interaction studies (http://www.fda.gov/cder/guidance) to create a more consistent evaluation approach. Methods and decision trees in the guidance on botanical drug products [171] pale in comparison. As stated previously, the experimental design aimed to quantify activity of a dietary substance should be determined by the drug(s)/probe substrate(s) and relevant phytochemical constituent(s) of interest. Data generated from well-designed *in vitro* studies (*e.g.*, K_m, V_{max}, K_i, IC_{50} determinations), combined with clinical PK information, may serve as a screening mechanism to rule out the need for additional *in vivo* studies, or provide a mechanistic basis for proper design of clinical studies using a modeling and simulation approach (see Section 5.1.4).

5.1.3. Development and validation of PK-PD relationships

Pharmacodynamic consequences of dietary substance-drug interactions are underexplored aspects of controlled clinical PK studies. Selection of one or more clinically relevant PD measures begins with a drug of interest for use in a target patient population. Most PD endpoints are surrogates

that correlate with clinical efficacy and are assessed in multiple dosing studies conducted over a period of more than one week or months. For example, antihypertensive drug trials measure blood pressure, antihistamine studies measure skin wheal formation, antiviral agent studies measure viral load, and opioid analgesic trials record pain scores (efficacy) and pupil diameter (toxicity) [172]. Depending on the therapeutic drug category, some PD measures have been validated over time and are well-accepted surrogates for drug efficacy and toxicity. Most food-drug interaction trials involve single dosing of healthy volunteers, who may exhibit different PK and PD outcomes compared to diseased patients. More studies in patient populations are needed to determine the short- and long-term responses to dietary substance-drug interactions.

A recent study with GFJ and atorvastatin demonstrated the importance of measuring PD outcomes and cautious extrapolation of conclusions from short term, healthy subject trials to the actual clinical setting of drug-treated patients [55]. Previous healthy volunteer studies have shown that large quantities (200-250 mL three times daily for ≥ two days) of GFJ increase exposure to atorvastatin by up to three-fold [173-175]. The Grapefruit Effect on Atorvastatin Therapy (GREAT) trial evaluated the effects of a typical daily single 'dose' of GFJ on plasma concentrations and lipid-lowering effects of atorvastatin in hyperlipidemic patients on a stable atorvastatin regimen (>90 days) [55]. Patients receiving extended treatment with atorvastatin (10, 20 or 40 mg daily) at a stable dose were randomized to two arms and received normal strength GFJ (300 mL daily) for 90 days. One cohort (Arm A, n = 60) continued on their current dose of atorvastatin; the other cohort (Arm B, n = 70) reduced the daily dose by half to confirm the need for atorvastatin dosage reduction while on GFJ. Serum atorvastatin, lipid profile, liver function, and creatine phosphokinase (to monitor for muscle toxicity) were measured at baseline and at 30, 60, and 90 days after starting GFJ. A statistically significant increase (19-26%) in median serum atorvastatin concentration was observed in Arm A, but changes in lipid profile were negligible. No adverse effects in liver or muscle were evident. Arm B showed a decrease (12-25%) in median serum atorvastatin with a small but statistically significant unfavorable serum lipid profile (*i.e.*, increased cholesterol, triglycerides, low-

density lipoprotein). No adverse effects on liver function tests or creatine phosphokinase were observed. Although the study results suggested chronic GFJ co-ingestion with atorvastatin is safe, several considerations should be noted. First, the lipid profile of the study population at baseline (*i.e.*, already on stable dose of atorvastatin) was reasonably controlled, so the addition of GFJ might have been minimally effective. Patients newly prescribed atorvastatin may have a different outcome; that is, concomitant GFJ may improve the lipid profile more rapidly and/or to a greater extent than in patients stabilized on atorvastatin. Second, the lack of effect on lipids may have been due to insufficient furanocoumarin content in the test juice; the increase in atorvastatin exposure was modest. Furanocoumarin content in the test GFJ was not reported, and whether it was measured is unclear. Third, the 100% Florida GFJ used in the study was from one lot and was tested for uniformity and nutritional content (*e.g.*, sugar, total carbohydrate, vitamin C). It is possible that the daily intake of carbohydrate and sugar in the GFJ offset some of the clinical benefit.

The aforementioned study highlights the importance of designing clinical studies that reflect typical eating/drinking habits. The study also draws attention to the potential use of dietary substances as 'PK boosters.' Indeed, deliberate inhibition of enteric CYP3A by GFJ consumption and/or individual components has been used by the scientific community for both therapeutic and pharmacoeconomic purposes. For example, this strategy has been evaluated and suggested to be beneficial to patients undergoing treatment for cancer and organ transplantation. Oral bioavailability would be increased without GFJ itself exerting additional adverse effects. Costs 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. The tyrosine kinase inhibitor imatinib, as well as the immunosuppressants sirolimus and tacrolimus, have been investigated clinically [56, 176, 177]. A deficiency common to all the studies is the lack of phytochemical analysis of the juice. The clinical trial investigating the effect of GFJ on sirolimus PK in advanced solid tumor patients initially showed no effect of GFJ, which was attributed to insufficient furanocoumarin content in the selected GFJ. A more 'potent' GFJ containing (unreported) inhibitory concentrations of furanocoumarins increased

plasma sirolimus concentrations by up to 400% relative to water. The clinical study evaluating the effect GFJ on imatinib PK in Japanese men diagnosed with chronic myelogenous leukemia showed no effect; furanocoumarin content in the GFJ was not measured. The lack of effect in the imatinib study could be attributed to the same reason a lack of effect was observed initially in the sirolimus study. A recent study in rats suggested that GFJ may be equally effective as ritonavir in increasing the bioavailability of the HIV protease inhibitor lopinavir [178]. Again, like the aforementioned studies, the GFJ was not analyzed for furanocoumarin content. In addition, translation of this interaction to the clinical setting has not been determined. Phytochemicals (*e.g.*, green tea catechins) capable of inhibiting efflux transporters have been studied with anti-tumor agents as multidrug resistance-reversing co-therapies [179, 180]. Long-term safety and pharmacoeconomic impact of combining drugs and dietary substances have not been evaluated sufficiently to change current disease management.

PD-related conclusions are unwarranted due to insufficient data on the PK relationship between the causative ingredient(s) and the drug(s). A rigorous evaluation of a PK-PD relationship can be achieved by applying *in silico* methods to *in vitro* and *in vivo* data. Modeling and simulation techniques permit the prediction of a potential interaction, but extensive *in vitro* data (*e.g.*, physicochemical properties, ADME parameters) and clinical PK information (including external datasets for validation) are imperative.

5.1.4. Prediction of food-drug interactions

PK-PD modeling and simulation have become powerful, integral tools for improving the efficiency of the drug development process, and the value of these approaches has been championed in several reviews [181-184]. The (patho)physiology of biological systems and the pharmacology of treatments acting on these systems can be modeled to predict quantitatively the dose-response relationship. Similar principles can be applied to dietary substances, especially in relation to how they interact with drugs. A quantitative analysis of both *in vitro* and clinical PK data is accomplished by a variety of algorithms and models (*e.g.*, basic, mechanistic, static, or dynamic) [185]. The application

of physiologically-based pharmacokinetic (PBPK) modeling to drug development has evolved over the past 10 years, reflecting significant advances in the predictability of key PK parameters from *in vitro* and *in vivo* data and in the availability of specialized software (*e.g.*, GastroPlus, Simcyp, Berkeley-Madonna, MATLAB-simulink) [186-190]. Framing the right question and capturing key assumptions are essential to delivering meaningful results. For the purpose of this review, modeling and simulation strategies as they apply to the quantitative prediction of CYP3A-mediated oral DDIs are discussed. Lessons from these approaches can be applied to dietary substance-drug interactions to establish a framework for the quantitative prediction of such interactions (Table 1.6).

The utility and accuracy of *in vitro* CYP inhibition data in the prediction of *in vivo* DDIs has been examined in numerous works with varying degrees of success [191-206]. Most of the cited predictive models are related to hepatic CYP3A-mediated interactions since they are well-characterized and >50% of marketed pharmaceutical agents are CYP3A substrates, allowing for validation. However, models have been modified to incorporate the contribution from the intestine, as studies demonstrated that inhibition of *both* hepatic and intestinal metabolism was needed for an improved DDI prediction [207-215]. PBPK modeling is used extensively, and such a data-driven method relies on extensive information on the victim *and* precipitant drugs – physicochemical properties, intestinal permeability, organ clearance processes, etc. The difficulty with dietary substances is the paucity of research on their oral bioavailability, metabolic mechanisms, human PK, and concentrations achieved at the site(s) of action. Despite the challenges, evaluation of a suspected dietary substance-drug interaction is possible, and one particularly relevant to this discussion is described [216].

Furanocoumarins in GFJ have been studied extensively over the past two decades as inhibitors of CYP3A4 (intestinal *and* hepatic, although the latter is less relevant). Bergamottin and 6',7'-dihydroxybergamottin (DHB) are typically the most abundant furanocoumarins present in GFJ and have been proposed as major intestinal CYP3A4 inhibitors contributing to GFJ-drug interactions [47, 48]. These compounds have been characterized to the extent that they can be considered 'marker'

compounds. Quantification of a given juice in terms of furanocoumarin content could be used to predict the likelihood and magnitude of an interaction with a CYP3A substrate and make between-study comparisons. Modeling and simulation can be used to validate one or more marker furanocoumarins as predictors of an interaction. As discussed previously, given the possibility of using GFJ and/or individual constituents as a 'drug-sparing, PK-boosting agent,' a standardized approach to evaluate quantitatively GFJ-drug interactions is imperative for the proper clinical management of patients.

A PBPK modeling approach was used to predict the impact of inhibiting intestinal and hepatic metabolism on human PK of CYP3A substrates [216]. One of the inhibitors of interest was DHB, which was given in the form of GFJ in two clinical studies [44, 217]. The objective was to predict the PK of simvastatin and midazolam in the presence of DHB in humans. A model was developed (software not specified) to account for absorption (gut), distribution, and hepatic clearance of the substrates and DHB. An important set of data to calculate parameters related to those processes is physicochemical properties of the drugs and DHB. Molecular structure, solubility, and permeability properties have been used to develop *in silico* models that allow the early estimation of several ADME properties. Commercial ADME software programs are reviewed elsewhere [218]. Inhibitory parameters of DHB (*i.e.*, K_i and k_{inact}) measured in human intestinal microsomes and unbound DHB concentration in human intestinal microsomes were incorporated into the model. Because no plasma concentration-time profile of DHB was available (or detectable), the concentration of DHB in the gut membrane was simulated using the PBPK model. For the simulations, the authors assumed a DHB amount of 43 µmol. Interestingly, DHB was not measured in either of the GFJs used in the clinical studies. Nevertheless, the resulting profile was used to predict the inhibitory effect of GFJ on midazolam and simvastatin PK.

AUC and maximum plasma concentration (C_{max}) were predicted successfully within a two-fold error range either in the absence or presence of DHB. This PBPK study was the first to investigate the impact of an inhibitory food ingredient on drug PK. Additional studies with other

substrates would validate this approach. Once validated, the techniques can be applied to other food ingredients. Although the aforementioned study was specific to CYP3A, it is important to note that food and substances in food can inhibit both uptake and efflux transporters in the intestine; the final result on drug bioavailability will depend on the more important contributor to absorption. Furthermore, the interplay between CYPs and transporters in dietary substance-drug interactions is recognized [219]; thus, incorporation of more complex processes will require more detailed data. Literature examples for modeling/predicting intestinal transporter-mediated interactions are few [201, 220].

In summary, whole body physiology is described by a series of linked mathematical equations with model parameters corresponding to measurable quantities, such as blood flow rates and tissue volumes. *In vitro* data relevant to drug absorption, distribution, metabolism, and excretion are scaled to *in vivo* scenarios. The new FDA guidance on DDI studies emphasizes the use of modeling and simulation to streamline the development process. Although such an approach is not required of those in the food/dietary supplement industries, modeling and simulation of new drug candidates and known dietary ingredients should be undertaken. This approach may rectify a considerable amount of work that may otherwise be inconsistent, contradictory, and irreproducible.

6. Conclusions

Interactions between medications and dietary substances, as foods or supplements, remain a relatively understudied and misunderstood area of pharmacotherapy. The upward trend of polypharmacy and ever-increasing consumer perception, rather misconception, of "all-natural" (*i.e.*, safe) remedies from dietary supplements and so-called 'superfood' sources has contributed to the potential for dangerous interactions. Although significant progress has been made in understanding mechanisms of intestinal inhibition by CYPs and efflux/uptake transport proteins, questions remain. Results from *in vitro* studies have not always translated to the clinic; these in *vitro-in vivo* discordances may be due to a lack of well-designed, proof-of-concept studies that control for as many confounding factors as possible. The complex nature of dietary substances and lack of adequate

characterization preclude between-study comparisons, as well as accurate predictions of drug interaction liability.

Since no ‘standard’ system exists to predict the effect of dietary substances on drug disposition, researchers in the field can model their scientific approach after that used in drug development. Integration of data from *in vitro*, *in vivo*, and *in silico* studies can optimize study designs and clarify potential risks of inhibition of intestinal metabolism/transport by a given dietary substance on pharmacotherapeutic outcomes. Such an aggressive assessment requires a multidisciplinary collaboration of experts from several fields, including clinical pharmacologists, pharmacognosists/natural products chemists, and botanists [159, 221]. The ultimate goal is to develop practice guidelines to provide a consistent approach in managing drug-dietary substance interactions appropriately.

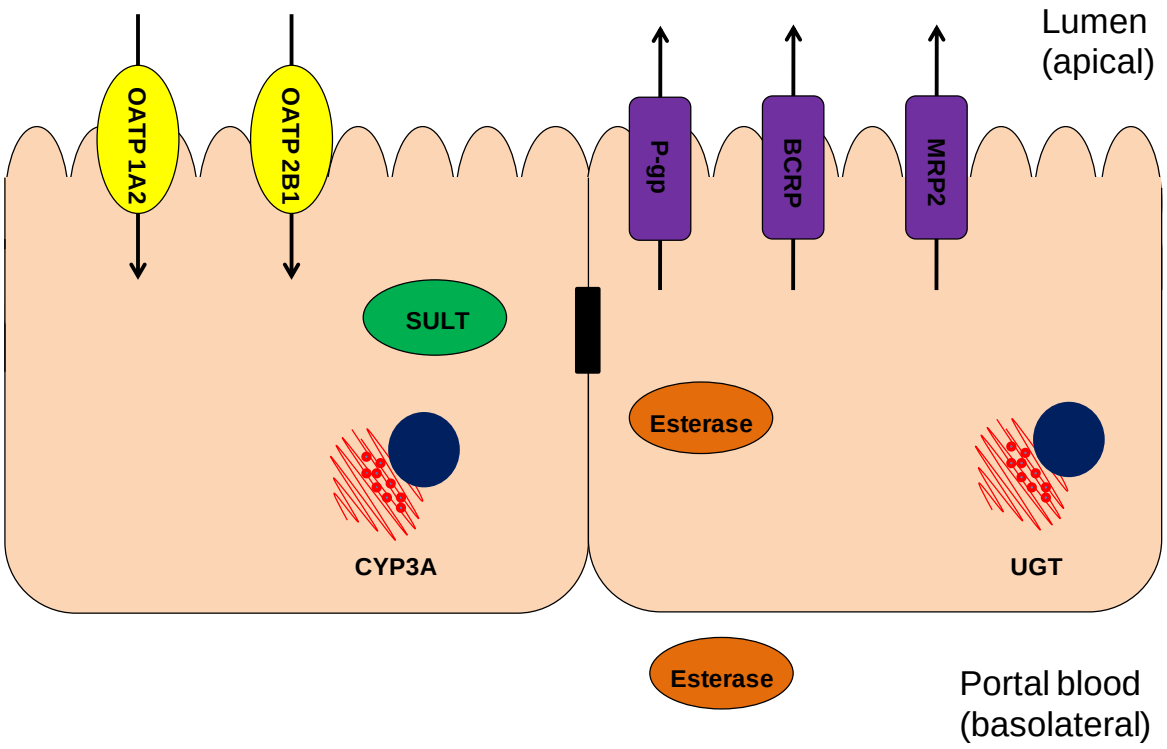

Figure 1.2. Schematic representation of enterocytes. Like drugs, dietary substances can alter systemic "victim" drug exposure by inhibiting enteric transporter-mediated uptake and/or efflux, as well as phase I and II metabolism. BCRP: breast cancer resistance protein, CYP: cytochrome P450, MRP: multidrug resistance-associated protein, OATP: organic anion-transporting polypeptide, P-gp: P-glycoprotein, SULT: sulfotransferase, UGT: UDP-glucuronosyltransferase.

Table 1.3. Summary of recent* controlled clinical studies involving CYP3A-mediated citrus juice-drug interactions

Citrus Juice	Subjects (n)	Administration Regimen of Drug and Juice Product (Manufacturer)	Change in Mean AUC	Reference
Grapefruit	Healthy volunteers (12)	*S*-ketamine 0.2 mg/kg x 1 on Day 5 Normal strength (Greippi Täysmehu; Valio Ltd., Helsinki, Finland) Pre-treament: 200 mL tid x 4 days Day 5: 150 mL with *S*-ketamine, 200 mL x 2 per pre-treatment schedule	↑ 185%[†] ($p < 0.001$)	51
	Addison's disease patients (17)	Cortisone acetate[#] 6.3 to 25 mg bid or tid per patient's prescribed regimen Normal strength (Meierienes Premium Rosa Grapefruktjuice; Tine SA, Oslo, Norway) 200 mL tid with cortisone acetate x 2 days Day 3: 200 mL with cortisone acetate	Cortisol: ↑ 19%[‡] ($p < 0.05$) Cortisone: ↑ 8.6%[‡] (NS)	52
	Healthy volunteers (20)	Tolvaptan 60 mg x 1 Single strength (NSP) 240 mL x 1 with tolvaptan	↑ 73%[§] (NC)	53
	Healthy volunteers (21)	Colchicine 0.6 mg x 1 on Day 4 "Undiluted" (NSP) Pre-treatment: 240 mL bid x 3 days Day 4: 240 mL with colchicine, 240 mL x 1 per pre-treatment schedule	↑ 2.7%[†] (NS)	54
	Hyperlipidemic patients (130; Arm A: 60, Arm B: 70)	Atorvastatin 10 to 40 mg daily per patient's prescribed regimen (Arm A) Atorvastatin 5 to 20 mg daily per patient's prescribed regimen (Arm B) Normal strength (NSP, Florida) 300 mL with atorvastatin x 90 days	Arm A: ↑ 19%[^] ($p < 0.05$) Arm B: ↓ 26%[^] ($p<0.001$)	55
	Chronic myelogenous leukemia patients (4)	Imatinib 400 mg daily Normal strength (Tropicana; Kirin, Tokyo, Japan) 250 mL with imatinib x 7 days	↑ 1.6%[¶] ($p = 0.715$)	56

Seville Orange	Healthy volunteers (23)	Colchicine 0.6 mg x 1 on Day 4 "Undiluted" (NSP) Pre-treatment: 240 mL bid x 3 days Day 4: 240 mL with colchicine, 240 mL x 1 per pre-treatment schedule	↓ 21% (SS)	54

*published since August 2010

[†]geometric mean AUC

[#]Cortisone acetate administered orally is converted to cortisol by hepatic 11β-hydroxysteroid dehydrogenase type 1. Circulating cortisol and cortisone are metabolized mainly by 5α/β-reductases, but CYP3A4 also may contribute.

[‡]median AUC

[§]n = 15

[^]median serum concentration at Day 90 relative to baseline

[¶]median peak concentration

tid, three times a day; bid, two times a day; NS, not statistically significant; NSP, not specified; NC, not calculated; SS, statistically significant as reflected by 90% confidence interval outside 80-125% range (*i.e.*, no interaction range)

Table 1.4. Potential explanations for lack of concordance between *in vitro* and *in vivo* dietary substance-drug interaction studies

- Lack of authentication and/or misidentification of source material
- Inadequate description of raw materials
  - Commercially available: brand name, manufacturer, lot number, ingredients, preparation directions, manufacturing process, origins of growth and production
  - Freshly prepared: scientific name, quantity, plant part used, site of collection, preparation procedures, storage conditions
- Adulteration of product (*e.g.*, contamination with other substances)
- Use of inconsistent product brand and lots
- Insufficient analysis (*e.g.*, physicochemical properties, biochemical activity, *in vivo* PK) of active constituents and/or metabolites in each batch being studied by a validated analytical method
- Species differences in metabolism and transport pathways
  - Pharmacogenetic variations
- Poorly designed *in vitro* experiments related to cell systems, assay conditions (*e.g.*, enzyme/transport protein probe substrate(s), negative and positive controls, proper concentrations of inhibitors and/or inducers), analytical methods
- Suboptimal clinical trial design related to adequate number of subjects (to achieve statistical power), dietary restriction(s) prior to and/or during study participation, randomization, blinding, placebo, positive controls, selection of enzyme/transport protein probe substrate, inhibitor and/or inducer dose(s) and dosing schedule, sampling times
- Scarce information on PK-PD relationship of dietary substance or specific active ingredients
- Extrapolation of conclusions based on one preparation

Adapted from [4] and [157]

Table 1.5. Questions to consider when reviewing clinical dietary substance-drug interaction studies

1. For a commercially available product, were the following provided – brand name, manufacturer, lot number, ingredients on label, preparation and storage directions, manufacturing process, origin(s) of growth and production?
2. Were any relevant/suspected bioactive constituent(s) measured by a validated analytical method?
3. Was the sample size justified by a power calculation?
4. If assay sensitivity was not an issue, were the sampling times appropriate (*i.e.*, full PK profile captured)?
5. For single or multiple dosing of the drug or dietary substance, was the given dose a typically consumed/recommended/prescribed dose (*i.e.*, reflective of 'real world' situations)?
6. Were dietary restrictions imposed on the subjects during the study period? Was a diet history taken prior to and/or during the study period?
7. Was pharmacogenetics considered as a source of variability?

Table 1.6. General framework for quantitative prediction of dietary substance-drug interactions involving CYP inhibition as a major underlying mechanism

PBPK Modeling Tools*	acslX, MATLAB-simulink, ADAPT, Berkeley-Madonna, MCSIM, SAAM II, GastroPlus, PK-Sim, Simcyp
1. Characterization of physicochemical properties[†] of drug and phytochemical inhibitor	pK_a, log P, f_u plasma, B/P ratio, P_{eff}[#], solubility[#]
2. Identification and quantification of clearance pathway(s) based on *in vitro* and/or *in vivo* studies - liver/intestine/kidney/etc.: CYP, non-CYP, transport	K_m, V_{max}, Cl_{int}, K_i f_m k_{inact}, k_{deg}, k_{syn}
3. Model-building based on information from Steps 1 and 2	Physiological parameters: organ blood flows, tissue partition coefficients (K_p)[†] Scaling factors (microsomes, primary cells → organ)
4. Simulation of plasma concentration-time profile(s) of drug	C_{max}, AUC, Cl, Cl/F
5. Comparison of simulated plasma concentration-time profile(s) with observed *in vivo* PK data	Visual predictive checks Prediction fold-error
6. Model refinement based on comparison of estimated parameters with those obtained from *in vivo* PK - Conduct sensitivity analysis if necessary	
7. Simulation of plasma concentration-time profile(s) of phytochemical inhibitor	C_{max}, AUC, Cl, Cl/F
8. Comparison of simulated plasma concentration-time profile(s) with observed *in vivo* PK data[‡]	Visual predictive checks Prediction fold-error
9. Model refinement based on comparison of estimated parameters with those obtained from *in vivo* PK	
10. Simulation of plasma concentration-time profile(s) of drug with phytochemical inhibitor	Drug: AUC_i/AUC

*Not all-inclusive list

[#]BCS/BCDDS classification

[†]Measured experimentally or predicted using software

[‡]Not always measurable

B/P, blood to plasma ratio; Cl_{int}, intrinsic clearance; f_m, fraction metabolized; f_u, fraction unbound in plasma; P_{eff}, effective intestinal permeability; k_{deg}, rate of degradation, k_{inact}, rate of inactivation; k_{synth}, rate of synthesis; Cl/F, apparent oral clearance; AUC_i/AUC, ratio of drug AUC in presence of inhibitor to AUC without inhibitor

REFERENCES

[1] Charman, W. N., Porter, C. J., Mithani, S., & Dressman, J. B. (1997). Physiochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH. *J Pharm Sci 86*, 269-282.

[2] Custodio, J. M., Wu, C. Y., & Benet, L. Z. (2008). Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Adv Drug Deliv Rev 60*, 717-733.

[3] Rodríguez-Fragoso, L., Martínez-Arismendi, J. L., Orozco-Bustos, D., Reyes-Esparza, J., Torres, E., & Burchiel, S. W. (2011). Potential risks resulting from fruit/vegetable-drug interactions: effects on drug-metabolizing enzymes and drug transporters. *J Food Sci 76*, R112-R124.

[4] Farkas, D. & Greenblatt, D. J. (2008). Influence of fruit juices on drug disposition: discrepancies between in vitro and clinical studies. *Expert Opin Drug Metab Toxicol, 4*, 381-393.

[5] Abdel-Rahman, A., Anyangwe, N., Carlacci, L., Casper, S., Danam, R. P., Enongene, E., *et al*. (2011). The safety and regulation of natural products used as foods and food ingredients. *Toxicol Sci 123*, 333-348.

[6] Santos, C. A. & Boullata, J. I. (2005). An approach to evaluating drug-nutrient interactions. *Pharmacotherapy 25*, 1789-1800.

[7] Genser, D. (2008). Food and drug interaction: consequences for the nutrition/health status. *Ann Nutr Metab 52 (Suppl 1)*, 29-32.

[8] Fleisher, D., Li, C., Zhou, Y., Pao, L. H., & Karim, A. (1999). Drug, meal and formulation interactions influencing drug absorption after oral administration. Clinical implications. *Clin Pharmacokinet 36*, 233-254.

[9] Li, Z., Vachharajani, N. N., & Krishna, R. (2002). On the assessment of effects of food on the pharmacokinetics of drugs in early development. *Biopharm Drug Dispos 23*, 165-71.

[10] Lentz, K. A. (2008). Current methods for predicting human food effect. *AAPS J 10*, 282-288.

[11] Parrott, N., Lukacova, V., Fraczkiewicz, G., & Bolger, M. B. (2009). Predicting pharmacokinetics of drugs using physiologically based modeling-application to food effects. *AAPS J 11*, 45-53.

[12] Singh BN. (1999). Effects of food on clinical pharmacokinetics. *Clin Pharmacokinet 37*, 213-255.

[13] Lourenço, R. (2001). Enteral feeding: drug/nutrient interaction. *Clin Nutr 20*, 187-193.

[14] Jung, H., Peregrina, A. A., Rodriquez, J. M., & Moreno-Esparza, R. (1997). The influence of coffee with milk and tea with milk on the bioavailability of tetracycline. *Biopharm Drug Dispos 18*, 459-463.

[15] Polk, R. E. (1989). Drug-drug interactions with ciprofloxacin and other fluoroquinolones. *Am J Med 87*(5A), 76S-81S.

[16] Plosker, G. L. & Scott, L. J. (2003). Saquinavir: a review of its use in boosted regimens for treating HIV infection. *Drugs 63*, 1299-1324.

[17] Le Tiec, C., Barrail, A., Goujard, C., & Taburet, A. M. (2005). Clinical pharmacokinetics and summary of efficacy and tolerability of atazanavir. *Clin Pharmacokinet 44*, 1035-1050.

[18] Wolinsky, I. & Williams, L. (2002). Nutrition in pharmacy practice. Washington, DC: American Pharmaceutical Association.

[19] McCabe, B. J., Frankel, E. H., & Wolfe, J. J. (2003). Handbook of food-drug interactions. Florida: CRC Press.

[20] Boullata, J. I. & Armenti, V. T. (2004). Handbook of drug-nutrient interactions. New Jersey: Humana.

[21] Meckling, K. A. (2007). Nutrient-drug interactions. Florida: CRC Press.

[22] Stargrove, M. B., Treasure, J., & McKee, D. L. (2008). Herb, nutrient, and drug interactions: clinical implications and therapeutic strategies. Missouri: Mosby Elsevier.

[23] Chan, L. N. (2002). Drug-nutrient interaction in clinical nutrition. *Curr Opin Clin Nutr Metab Care 5*, 327-332.

[24] Holbrook, A. M, Pereira, J. A., Labiris, R., McDonald, H., Douketis, J. D., Crowther, M., *et al*. (2005). Systematic overview of warfarin and its drug and food interactions. *Arch Intern Med 165*, 1095-1106.

[25] Brown, C., Taniguchi, G., Yip, K. (1989). The monoamine oxidase inhibitor-tyramine interaction. *J Clin Pharmacol 29*, 529-532.

[26] Self, T. H., Chrisman, C. R., Baciewicz, A. M., & Bronze, M. S. Isoniazid drug and food interactions. *Am J Med Sci 317*, 304-311.

[27] Pulido, R., Hernández-García, M., & Saura-Calixto, F. (2003). Contribution of beverages to the intake of lipophilic and hydrophilic antioxidants in the Spanish diet. *Eur J Clin Nutr 57*, 1275-1282.

[28] Kaplan, N. M. & Palmer, B. F. (2000). Nutritional and health benefits of beer. *Am J Med Sci 320*, 320-326.

[29] Guilford, J. M. & Pezzuto, J. M. (2011). Wine and health: a review. *Am J Enol Vitic 62*, 471-486.

[30] Tidball, C. S. (1971). The nature of the intestinal epithelial barrier. *Am J Dig Dis 16*, 745-767.

[31] McCabe, B. J. (2004). Prevention of food-drug interactions with special emphasis on older adults. *Curr Opin Clin Nutr Metab Care 7*, 21-26.

[32] Espin, J. C., Garcia-Conesa, M. T., & Tomas-Barberan, F. A. (2007). Nutraceuticals: facts and fiction. *Phytochemistry 68*, 2986-3008.

[33] Gruenwald, J. (2009). Novel botanical ingredients for beverages. *Clin Dermatol 27*, 210-216.

[34] Huang, S. M., Strong, J. M., Zhang, L., Reynolds, K. S., Nallani, S., Temple, R., *et al*. (2008). New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. *J Clin Pharmacol 48*, 662-670.

[35] Shen, D. D., Kunze, K. L., & Thummel, K. E. (1997). Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv Drug Deliv Rev 27*, 99-127.

[36] Lin, J. H. & Lu, A. Y. (2001). Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu Rev Pharmacol Toxicol 41*, 535-567.

[37] Paine, M. F., Hart, H. L., Ludington, S. S., Haining, R. L., Rettie, A. E., & Zeldin, D. C. (2006). The human intestinal cytochrome P450 "pie". *Drug Metab Dispos 34*, 880-886.

[38] Thummel, K. E & Wilkinson, G. R. (1998). In vitro and in vivo drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol 38*, 389-430.

[39] Gibbs, M. A. & Hosea, N. A. (2003). Factors affecting the clinical development of cytochrome p450 CYP3A substrates. *Clin Pharmacokinet 42*, 969-984.

[40] Mertens-Talcott, S. U., Zadezensky, I., De Castro, W. V., Derendorf, H., & Butterweck, V. (2006). Grapefruit-drug interactions: can interactions with drugs be avoided? *J Clin Pharmacol 46*, 1390-1416.

[41] Seden, K., Dickinson, L., Khoo, S., Back, D. (2010). Grapefruit-drug interactions. *Drugs 70*, 2373-2407.

[42] Hanley, M. J., Cancalon, P., Widmer, W. W., & Greenblatt, D. J. (2011). The effect of grapefruit juice on drug disposition. *Expert Opin Drug Metab Toxicol 7*, 267-286.

[43] Paine, M. F. & Oberlies, N. H. (2007). Clinical relevance of the small intestine as an organ of drug elimination: drug-fruit juice interactions. *Expert Opin Drug Metab Toxicol 3*, 67-80.

[44] Kupferschmidt, H. H., Ha, H. R., Ziegler, W. H., Meier, P. J., & Krahenbuhl, S. (1995). Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther 58*, 20-28.

[45] Saito, M., Hirata-Koizumi, M., Matsumoto, M., Urano, T., & Hasegawa, R. (2005). Undesirable effects of citrus juice on the pharmacokinetics of drugs: focus on recent studies. *Drug Saf 28*, 677-694.

[46] Schmiedlin-Ren, P., Edwards, D. J., Fitzsimmons, M. E., He, K., Lown, K. S., Woster, P. M., *et al*. (1997). Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. *Drug Metab Dispos 25*, 1228-1233.

[47] Paine, M. F., Criss, A. B., & Watkins, P. B. (2004). Two major grapefruit juice components differ in intestinal CYP3A4 inhibition kinetic and binding properties. *Drug Metab Dispos 32*, 1146-1153.

[48] Paine, M. F, Criss, A. B., & Watkins, P. B. (2005). Two major grapefruit juice components differ in time to onset of intestinal CYP3A4 inhibition. *J Pharmacol Exp Ther 312*, 1151-1160.

[49] Lown, K. S., Bailey, D. G., Fontana, R. J., Janardan, S. K., Adair, C. H., Fortlage, L. A., *et al*. (1997). Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest 99*, 2545-2553.

[50] Won, C. S., Oberlies, N. H., & Paine, M. F. Influence of dietary substances on intestinal drug metabolism and transport. *Curr Drug Metab 11*, 778-792.

[51] Peltoniemi, M. A., Saari, T. I., Hagelberg, N. M., Laine, K., Neuvonen, P. J., Olkkola, K. T. (2012). *S*-ketamine concentrations are greatly increased by grapefruit juice. *Eur J Clin Pharmacol* DOI: 10.1007/s00228-012-1214-9.

[52] Methlie, P., Husebye, E. E., Hustad, S., Lien, E. A., & Løvås, K. (2011). Grapefruit juice and licorice increase cortisol availability in patients with Addison's disease. *Eur J Endocrinol 165*, 761-769.

[53] Shoaf, S. E., Mallikaarjun, S., & Bricmont, P. Effect of grapefruit juice on the pharmacokinetics of tolvaptan, a non-peptide arginine vasopressin antagonist, in healthy subjects. *Eur J Clin Pharmacol 68*, 207-211.

[54] Wason, S., DiGiacinto, J., & Davis, M. W. (2011). Consumption of grapefruit or Seville orange juice does not affect the pharmacokinetics of colchicine. *American Society for Clinical Pharmacology and Therapeutics* Annual Meeting Abstract PI-77, Dallas, TX, March 2-5, *2011*, American Society for Clinical Pharmacology and Therapeutics.

[55] Reddy, P., Ellington, D., Zhu, Y., Zdrojewski, I., Parent, S. J., Harmatz, J. S., *et al*. (2011). Serum concentrations and clinical effects of atorvastatin in patients taking grapefruit juice daily. *Br J Clin Pharmacol 72*, 434-441.

[56] Kimura, S., Kako, S., Wada, H., Sakamoto, K., Ashizawa, M., Sato, M., *et al*. (2011). Can grapefruit juice decrease the cost of imatinib for the treatment of chronic myelogenous leukemia? *Leuk Res 35*, e11-12.

[57] Valenzuela, B., Rebollo, J., Pérez, T., Brugarolas, A., & Pérez-Ruixo, J. J. (2011). Effect of grapefruit juice on the pharmacokinetics of docetaxel in cancer patients: a case report. *Br J Clin Pharmacol 72*, 978-981.

[58] Agosti S, Casalino L, Bertero G, Barsotti A, Brunelli C, & Morelloni S. (2012). A dangerous fruit juice. *Am J Emerg Med 30*, e5-8.

[59] Lilja, J. J., Kivistö, K. T., Backman, J. T., & Neuvonen, P. J. (2000). Effect of grapefruit juice dose on grapefruit juice-triazolam interaction: repeated consumption prolongs triazolam half-life. *Eur J Clin Pharmacol 56*, 411-415.

[60] Guo, L. Q., Fukuda, K., Ohta, T., & Yamazoe, Y. (2000). Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity. *Drug Metab Dispos 28*, 766-771.

[61] Malhotra, S., Bailey, D. G., Paine, M. F., Watkins, P. B. (2001). Seville orange juice-felodipine interaction: comparison with dilute grapefruit juice and involvement of furocoumarins. *Clin Pharmacol Ther 69*, 14-23.

[62] Edwards, D. J., Fitzsimmons, M. E., Schuetz, E. G., Yasuda, K., Ducharme, M. P., Warbasse, L. H., *et al*. (1999). 6',7'-Dihydroxybergamottin in grapefruit juice and Seville orange juice: effects on cyclosporine disposition, enterocyte CYP3A4, and P-glycoprotein. *Clin Pharmacol Ther 65*, 237-244.

[63] Penzak SR, Acosta EP, Turner M, Edwards DJ, Hon YY, Desai HD, *et al*. (2002). Effect of Seville orange juice and grapefruit juice on indinavir pharmacokinetics. *J Clin Pharmacol 42*, 1165-1170.

[64] Mouly, S. J., Matheny, C., Paine, M. F., Smith, G., Lamba, J., Lamba, V., *et al*. (2005). Variation in oral clearance of saquinavir is predicted by *CYP3A5*1* genotype but not by enterocyte content of cytochrome P450 3A5. *Clin Pharmacol Ther 78*, 605-618.

[65] Tateishi, T., Soucek, P., Caraco, Y., Guengerich, F. P., & Wood, A. J. (1997). Colchicine biotransformation by human liver microsomes. Identification of CYP3A4 as the major isoform responsible for colchicine demethylation. *Biochem Pharmacol 53*, 111-116.

[66] Tzulker, R., Glazer, I., Bar-Ilan, I., Holland, D., Aviram, M., & Amir, R. (2007). Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J Agric Food Chem 55*, 9559-9570.

[67] Hidaka, M., Okumura, M., Fujita, K., Ogikubo, T., Yamasaki, K., Iwakiri, T., *et al*. (2005). Effects of pomegranate juice on human cytochrome p450 3A (CYP3A) and carbamazepine pharmacokinetics in rats. *Drug Metab Dispos 33*, 644-648.

[68] Farkas, D., Oleson, L. E., Zhao, Y., Harmatz, J. S., Zinny, M. A., Court, M. H., *et al*. (2007). Pomegranate juice does not impair clearance of oral or intravenous midazolam, a probe for cytochrome P450-3A activity: comparison with grapefruit juice. *J Clin Pharmacol 47*, 286-294.

[69] Misaka S, Nakamura R, Uchida S, Takeuchi K, Takahashi N, Inui N, *et al*. (2011). Effect of 2 weeks' consumption of pomegranate juice on the pharmacokinetics of a single dose of midazolam: an open-label, randomized, single-center, 2-period crossover study in healthy Japanese volunteers. *Clin Ther 33*, 246-252.

[70] Sunaga, K., Ohkawa, K., Nakamura, K., Ohkubo, A., Harada, S., & Tsuda, T. (2012). Mechanism-based inhibition of recombinant human cytochrome P450 3A4 by tomato juice extract. *Biol Pharm Bull 35*, 329-334.

[71] Chun, A. H., Erdman, K., Chiu, Y. L., Pilmer, B. L., Achari, R., & Cavanaugh, J. H. (2002). Bioavailability of lansoprazole granules administered in juice or soft food compared with the intact capsule formulation. *Clin Ther 24*, 1322-1331.

[72] Dreosti, I. E. (1996). Bioactive ingredients: antioxidants and polyphenols in tea. *Nutr Rev 54*, S51-8.

[73] Vinson, J. A., Teufel, K., & Wu, N. (2004). Green and black teas inhibit atherosclerosis by lipid, antioxidant, and fibrinolytic mechanisms. *J Agric Food Chem 52*, 3661-3665.

[74] Sang, S., Lambert, J. D., Ho, C. T., & Yang, C. S. (2011). The chemistry and biotransformation of tea constituents. *Pharmacol Res 64*, 87-99.

[75] Colalto, C. (2010). Herbal interactions on absorption of drugs: Mechanisms of action and clinical risk assessment. *Pharmacol Res 62*, 207-227.

[76] Antonello, M., Montemurro, D., Bolognesi, M., Di Pascoli, M., Piva, A., Grego, F., *et al*. (2007). Prevention of hypertension, cardiovascular damage and endothelial dysfunction with green tea extracts. *Am J Hypertens 20*, 1321-1328.

[77] Donovan, J. L., Chavin, K. D., Devane, C. L., Taylor, R. M., Wang, J. S., Ruan, Y., *et al*. (2004). Green tea (*Camellia sinensis*) extract does not alter cytochrome p450 3A4 or 2D6 activity in healthy volunteers. *Drug Metab Dispos 32*, 906-908.

[78] Chow, H. H., Hakim, I. A., Vining, D. R., Crowell, J. A., Cordova, C. A., Chew, W. M., *et al*. (2006). Effects of repeated green tea catechin administration on human cytochrome P450 activity. *Cancer Epidemiol Biomarkers Prev 15*, 2473-2476.

[79] Vischini, G., Niscola, P., Stefoni, A., & Farneti, F. (2011). Increased plasma levels of tacrolimus after ingestion of green tea. *Am J Kidney Dis 58*, 329.

[80] Qiao, J., Gu, C., Shang, W., Du, J., Yin, W., Zhu, M., *et al*. (2011). Effect of green tea on pharmacokinetics of 5-fluorouracil in rats and pharmacodynamics in human cell lines in vitro. *Food Chem Toxicol 49*, 1410-1415.

[81] Jang, G. R & Harris, R. Z. (2007). Drug interactions involving ethanol and alcoholic beverages. *Expert Opin Drug Metab Toxicol 3*, 719-731.

[82] Krenz, M. & Korthuis, R. J. (2012). Moderate ethanol ingestion and cardiovascular protection: from epidemiologic associations to cellular mechanisms. *J Mol Cell Cardiol 52*, 93-104.

[83] Bailey, D. G., Dresser, G. K., & Bend, J. R. (2003). Bergamottin, lime juice, and red wine as inhibitors of cytochrome P450 3A4 activity: comparison with grapefruit juice. *Clin Pharmacol Ther 73*, 529-537.

[84] Piver, B., Berthou, F., Dreano, Y., & Lucas, D. (2001). Inhibition of CYP3A, CYP1A and CYP2E1 activities by resveratrol and other non volatile red wine components. *Toxicol Lett 125*, 83-91.

[85] Stupans, L., Tan, H. W., Kirlich, A., Tuck, K., Hayball, P., & Murray, M. (2002). Inhibition of CYP3A-mediated oxidation in human hepatic microsomes by the dietary derived complex phenol, gallic acid. *J Pharm Pharmacol 54*, 269-275.

[86] Vinson, J. A., Mandarano, M., Hirst, M., Trevithick, J. R., & Bose, P. (2003). Phenol antioxidant quantity and quality in foods: beers and the effect of two types of beer on an animal model of atherosclerosis. *J Agric Food Chem 51*, 5528-5533.

[87] Huvaere, K., Andersen, M. L., Olsen, K., Skibsted, L. H., Heyerick, A., & De Keukeleire, D. (2003). Radicaloid-type oxidative decomposition of beer bittering agents revealed. *Chemistry 9*, 4693-4699.

[88] Henderson, M. C., Miranda, C. L., Stevens, J. F., Deinzer, M. L., & Buhler, D. R. (2000). In vitro inhibition of human P450 enzymes by prenylated fl avonoids from hops, *Humulus lupulus*. *Xenobiotica 30*, 235 -251.

[89] Foster, B. C., Kearns, N., Arnason, J. T., Saleem, A., Ogrodowczyk, C., & Desjardins, S. (2009). Comparative study of hop-containing products on human cytochrome p450-mediated metabolism. *J Agric Food Chem 57*, 5100-5105.

[90] Foster, B. C., Arnason, J. T., Saleem, A., Tam, T. W., Liu, R., Mao, J., *et al*. (2011). Comparative study of hops-containing products on human cytochrome P450-mediated metabolism. *J Agric Food Chem 59*, 5159-5163.

[91] Patchett, A. A. (1984). The chemistry of enalapril. *Br J Clin Pharmacol 18*, 201S-207S.

[92] Sabra, R. (1988). Topics in clinical pharmacology: lovastatin. *Am J Med Sci 296*, 426-428.

[93] Liederer, B. M. & Borchardt, R. T. (2006). Enzymes involved in the bioconversion of ester-based prodrugs. *J Pharm Sci 95*, 1177-1195.

[94] Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, *et al*. (2010). Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos 38*, 92-99.

[95] Clarke, T. A. & Waskell, L. A. (2003). The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin. *Drug Metab Dispos 31*, 53-59.

[96] Burchell, B., Brierley, C. H., & Rance, D. (1995). Specificity of human UDP-glucuronosyltransferases and xenobiotic glucuronidation. *Life Sci 57*, 1819-1831.

[97] Meech, R. & Mackenzie, P. I. (1997). Structure and function of uridine diphosphate glucuronosyltransferases. *Clin Exp Pharmacol Physiol 24*, 907-915.

[98] Radominska-Pandya, A., Czernik, P. J., Little, J. M., Battaglia, E., & Mackenzie, P. I. (1999). Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab Rev 31*, 817-899.

[99] Burchell, B., Brierley, C. H., Monaghan, G. and Clark, D. J. (1998). The structure and function of the UDP-glucuronosyltransferase gene family. *Adv Pharmacol 42*, 335-338.

[100] Ritter, J. K. (2007). Intestinal UGTs as potential modifiers of pharmacokinetics and biological responses to drugs and xenobiotics. *Expert Opin Drug Metab Toxicol 3*, 93-107.

[101] Harbourt, D. E., Fallon, J. K., Ito, S., Baba, T., Ritter, J. K., Glish, G. L., *et al*. (2012). Quantification of human uridine-diphosphate glucuronosyl transferase 1A isoforms in liver, intestine, and kidney using nanobore liquid chromatography-tandem mass spectrometry. *Anal Chem 84*, 98-105.

[102] van der Logt, E. M., Roelofs, H. M., Nagengast, F. M., & Peters, W. H. (2003). Induction of rat hepatic and intestinal UDP-glucuronosyltransferases by naturally occurring dietary anticarcinogens. *Carcinogenesis 24*, 1651-1656.

[103] Peterson, S., Bigler, J., Horner, N. K., Potter, J. D., Lampe, J. W. (2005). Cruciferae interact with the UGT1A1*28 polymorphism to determine serum bilirubin levels in humans. *J Nutr 135*, 1051-1055.

[104] Chang, J. L., Bigler, J., Schwarz, Y., Li, S. S., Li, L., King, I. B., *et al*. (2007). UGT1A1 polymorphism is associated with serum bilirubin concentrations in a randomized, controlled, fruit and vegetable feeding trial. *J Nutr 137*, 890-897.

[105] Fisher, M. B., Paine, M. F., Strelevitz, T. J., & Wrighton, S. A. (2001). The role of hepatic and extrahepatic UDP-glucuronosyltransferases in human drug metabolism. *Drug Metab Rev 33*, 273-297.

[106] Saracino MR, Bigler J, Schwarz Y, Chang JL, Li S, Li L, *et al*. (2009). Citrus fruit intake is associated with lower serum bilirubin concentration among women with the UGT1A1*28 polymorphism. *J Nutr 139*, 555-560.

[107] Navarro, S. L., Peterson, S., Chen, C., Makar, K. W., Schwarz, Y., King, I. B., *et al*. (2009). Cruciferous vegetable feeding alters UGT1A1 activity: diet- and genotype-dependent changes in serum bilirubin in a controlled feeding trial. *Cancer Prev Res (Phila) 2*, 345-52.

[108] Navarro, S. L., Chen, Y., Li, L., Li, S. S., Chang, J. L., Schwarz, Y., *et al*. (2011). UGT1A6 and UGT2B15 polymorphisms and acetaminophen conjugation in response to a randomized, controlled diet of select fruits and vegetables. *Drug Metab Dispos 39*, 1650-1657.

[109] Mohamed, M. E. & Frye, R, F. (2011). Inhibitory effects of commonly used herbal extracts on UDP-glucuronosyltransferase 1A4, 1A6, and 1A9 enzyme activities. *Drug Metab Dispos 39*, 1522-1528.

[110] Laverdière I, Caron P, Harvey M, Lévesque É, Guillemette C. (2011). In vitro investigation of human UDP-glucuronosyltransferase isoforms responsible for tacrolimus glucuronidation: predominant contribution of UGT1A4. *Drug Metab Dispos 39*, 1127-1130.

[111] Klaassen, C. D. & Boles, J. W. (1997) Sulfation and sulfotransferases 5: the importance of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J 11*, 404-418.

[112] Gamage, N., Barnett, A., Hempel, N., Duggleby, R. G., Windmill, K. F., Martin, J. L., *et al*. (2006). Human sulfotransferases and their role in chemical metabolism. *Toxicol Sci 90*, 5-22.

[113] Harris, R. M. & Waring, R. H. (2008). Sulfotransferase inhibition: potential impact of diet and environmental chemicals on steroid metabolism and drug detoxification. *Curr Drug Metab 9*, 269-275.

[114] Coughtrie, M. W. & Johnston, L. E. (2001). Interactions between dietary chemicals and human sulfotransferases-molecular mechanisms and clinical significance. *Drug Metab Dispos 29*, 522-528.

[115] Nishimuta H, Tsujimoto M, Ogura K, Hiratsuka A, Ohtani H, & Sawada Y. (2005). Inhibitory effects of various beverages on ritodrine sulfation by recombinant human sulfotransferase isoforms SULT1A1 and SULT1A3. *Pharm Res 22*, 1406-1410.

[116] Nishimuta, H., Ohtani, H., Tsujimoto, M., Ogura, K., Hiratsuka, A., & Sawada, Y. (2007). Inhibitory effects of various beverages on human recombinant sulfotransferase isoforms SULT1A1 and SULT1A3. *Biopharm Drug Dispos 28*, 491-500.

[117] Eagle, K. (2012). Toxicological effects of red wine, orange juice, and other dietary SULT1A inhibitors via excess catecholamines. *Food Chem Toxicol* DOI: 10.1016/j.fct.2012.03.004.

[118] Huang, S. M., Zhao, H., Lee, J. I., Reynolds, K., Zhang, L., Temple, R., *et al*. (2010). Therapeutic protein-drug interactions and implications for drug development. *Clin Pharmacol Ther 87*, 497-503.

[119] Benet, L. Z. (2009). The drug transporter-metabolism alliance: uncovering and defining the interplay. *Mol Pharm 6*, 1631-1643.

[120] Dresser, G. K., Bailey, D. G., Leake, B. F., Schwarz, U. I., Dawson, P. A., Freeman, D. J., *et al*. (2002). Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther 71*, 11-20.

[121] Hagenbuch, B. & Gui, C. (2008). Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica 38*, 778-801.

[122] Kim, R. B. (2003). Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. *Eur J Clin Invest 33*(Suppl 2), 1-5.

[123] Greenblatt, D. J. (2009). Analysis of drug interactions involving fruit beverages and organic anion-transporting polypeptides. *J Clin Pharmacol 49*, 1403-1407.

[124] Bailey, D. G. (2010). Fruit juice inhibition of uptake transport: a new type of food-drug interaction. *Br J Clin Pharmacol 70*, 645-655.

[125] Tapaninen, T., Neuvonen, P. J., Niemi, M. (2010). Grapefruit juice greatly reduces the plasma concentrations of the OATP2B1 and CYP3A4 substrate aliskiren. *Clin Pharmacol Ther 88*, 339-342.

[126] Rebello, S., Zhao, S., Hariry, S., Dahlke, M., Alexander, N., Vapurcuyan, A., *et al*. (2011). Intestinal OATP1A2 inhibition as a potential mechanism for the effect of grapefruit juice on aliskiren pharmacokinetics in healthy subjects. *Eur J Clin Pharmacol* DOI: 10.1007/s00228-011-1167-4.

[127] Ho, P. C., Saville, D. J., Coville, P. F., & Wanwimolruk, S. (2000). Content of CYP3A4 inhibitors,naringin, naringenin and bergapten in grapefruit and grapefruit juice products. *Pharm Acta Helv 74*, 379-385.

[128] Ross, S. A., Ziska, D. S., Zhao, K., ElSohly, M. A. (2000). Variance of common flavonoids by brand of grapefruit juice. *Fitoterapia 71*, 154-161.

[129] De Castro, W. V., Mertens-Talcott, S., Rubner, A., Butterweck, V., Derendorf, H. (2006). Variation of flavonoids and furanocoumarins in grapefruit juices: a potential source of variability in grapefruit juice-drug interaction studies. *J Agric Food Chem 54*, 249-255.

[130] Wanwimolruk, S. & Marquez, P. V. (2006). Variations in content of active ingredients causing drug interactions in grapefruit juice products sold in California. *Drug Metabol Drug Interact 21*, 233-243.

[131] Brill, S., Zimmermann, C., Berger, K., Drewe, J., Gutmann, H. (2009). In vitro interactions with repeated grapefruit juice administration-to peel or not to peel? *Planta Med 75*, 332-335.

[132] Parving, H. H., Brenner, B. M., McMurray, J. J., de Zeeuw, D., Haffner, S. M., Solomon, S. D., *et al*. (2009). Aliskiren trial in type 2 diabetes using cardio-renal endpoints (ALTITUDE): rationale and study design. *Nephrol Dial Transplant 24*, 1663-1671.

[133] Lilja, J. J., Backman, J. T., Laitila, J., Luurila, H., Neuvonen, P. J. (2003). Itraconazole increases but grapefruit juice greatly decreases plasma concentrations of celiprolol. *Clin Pharmacol Ther 73*, 192-198.

[134] Kato, Y., Miyazaki, T., Kano, T., Sugiura, T., Kubo, Y., Tsuji, A. (2009). Involvement of influx and efflux transport systems in gastrointestinal absorption of celiprolol. *J Pharm Sci 98*, 2529-2539.

[135] Ieiri, I., Doi, Y., Maeda, K., Sasaki, T., Kimura, M., Hirota, T., *et al*. (2011). Microdosing clinical study: pharmacokinetic, pharmacogenomic (SLCO2B1), and interaction (grapefruit juice) profiles of celiprolol following the oral microdose and therapeutic dose. *J Clin Pharmacol* DOI: 10.1177/0091270011408612.

[136] Sai, Y., Kaneko, Y., Ito, S., Mitsuoka, K., Kato, Y., Tamai, I., *et al*. (2006). Predominant contribution of organic anion transporting polypeptide OATP-B (OATP2B1) to apical uptake of estrone-3-sulfate by human intestinal Caco-2 cells. *Drug Metab Dispos 34*, 1423-1431.

[137] Hagenbuch, B. & Meier, P. J. (2003). The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta 1609*, 1-18.

[138] Mougey, E. B., Feng, H., Castro, M., Irvin, C. G., Lima, J. J. (2009). Absorption of montelukast is transporter mediated: a common variant of OATP2B1 is associated with reduced plasma concentrations and poor response. *Pharmacogenet Genomics 19*, 129-138.

[139] Mougey, E. B., Lang, J. E., Wen, X., Lima, J. J. (2011). Effect of citrus juice and SLCO2B1 genotype on the pharmacokinetics of montelukast. *J Clin Pharmacol 51*, 751-760.

[140] Chu, X., Philip, G., Evers, R. (2012). Comments on Mougey *et al*. (2009): Absorption of montelukast is transporter mediated: a common variant of OATP2B1 is associated with reduced plasma concentrations and poor response. *Pharmacogenet Genomics* 19: 129-138. *Pharmacogenet Genomics 22*, 319-322.

[141] Lilja, J. J., Raaska, K., Neuvonen, P. J. (2005). Effects of orange juice on the pharmacokinetics of atenolol. *Eur J Clin Pharmacol 61*, 337-340.

[142] Lilja, J. J., Juntti-Patinen, L., Neuvonen, P. J. (2004). Orange juice substantially reduces the bioavailability of the beta-adrenergic-blocking agent celiprolol. *Clin Pharmacol Ther 75*, 184-190.

[143] Neuhofel, A. L., Wilton, J. H., Victory, J. M., Hejmanowsk, L. G., Amsden, G. W. (2002). Lack of bioequivalence of ciprofloxacin when administered with calcium-fortified orange juice: a new twist on an old interaction. *J Clin Pharmacol 42*, 461-466.

[144] Wallace, A. W., Victory, J. M., Amsden, G. W. (2003). Lack of bioequivalence when levofloxacin and calcium-fortified orange juice are coadministered to healthy volunteers. *J Clin Pharmacol 43*, 539-544.

[145] Maeda, T., Takahashi, K., Ohtsu, N., Oguma, T., Ohnishi, T., Atsumi, R., *et al*. (2007). Identification of influx transporter for the quinolone antibacterial agent levofloxacin. *Mol Pharm 4*, 85-94.

[146] Tapaninen, T., Neuvonen, P. J., Niemi, M. (2011). Orange and apple juice greatly reduce the plasma concentrations of the OATP2B1 substrate aliskiren. *Br J Clin Pharmacol 71*, 718-726.

[147] Gerhauser, C. (2008). Cancer chemopreventive potential of apples, apple juice, and apple components. *Planta Med 74*, 1608-1624.

[148] Satoh, H., Yamashita, F., Tsujimoto, M., Murakami, H., Koyabu, N., Ohtani, H., *et al*. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos 33*, 518-523.

[149] Imanaga, J., Kotegawa, T., Imai, H., Tsutsumi, K., Yoshizato, T., Ohyama, T., *et al*. (2011). The effects of the SLCO2B1 c.1457C>T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenet Genomics 21*, 84-93.

[150] Ming, X., Knight, B. M., Thakker, D. R. (2011). Vectorial transport of fexofenadine across Caco-2 cells: involvement of apical uptake and basolateral efflux transporters. *Mol Pharm 8*, 1677-1686.

[151] Glaeser, H., Bailey, D. G., Dresser, G. K., Gregor, J. C., Schwarz, U. I., McGrath, J. S., *et al*. (2007). Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin Pharmacol Ther 81*, 362-370.

[152] Mandery, K., Bujok, K., Schmidt, I., Keiser, M., Siegmund, W., Balk, B., *et al*. (2010). Influence of the flavonoids apigenin, kaempferol, and quercetin on the function of organic anion transporting polypeptides 1A2 and 2B1. *Biochem Pharmacol 80*, 1746-1753.

[153] Jeon, H., Lim, K. S., Ohashi, K., Kotegawa, T., Ieiri, I., Cho, J. Y., *et al*. (2012). Apple juice greatly reduces plasma concentrations of atenolol. 2012 *American Society for Clinical Pharmacology and Therapeutics* Annual Meeting Abstract PII-78, National Harbor, MD, March 12-17, 2012, *American Society for Clinical Pharmacology and Therapeutics.*

[154] Zaveri, N. T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sci 78*, 2073-2080.

[155] Fuchikami, H., Satoh, H., Tsujimoto, M., Ohdo, S., Ohtani, H., Sawada, Y. (2006). Effects of herbal extracts on the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos 34*, 577-582.

[156] Roth, M., Timmermann, B. N., Hagenbuch, B. (2011). Interactions of green tea catechins with organic anion-transporting polypeptides. *Drug Metab Dispos 39*, 920-926.

[157] Glisson, J. K. & Walker, L. A. (2010). How physicians should evaluate dietary supplements. *Am J Med 123*, 577-582.

[158] Visioli, F., De La Lastra, C. A., Andres-Lacueva, C., Aviram, M., Calhau, C., Cassano, A., *et al*. (2011). Polyphenols and human health: a prospectus. *Crit Rev Food Sci Nutr 51*, 524-546.

[159] Dentali, S. J. (2010). Successful botanical research requires botanical expertise. *Clin Pharmacol Ther 87*, 149-151.

[160] Smillie, T. J. & Khan, I. A. (2010). A comprehensive approach to identifying and authenticating botanical products. *Clin Pharmacol Ther 87*, 175-186.

[161] Markowitz, J. S., von Moltke, L. L., Donovan, J. L. (2008). Predicting interactions between conventional medications and botanical products on the basis of in vitro investigations. *Mol Nutr Food Res 52*, 747-754.

[162] Kim, E., Sy-Cordero, A., Graf, T. N., Brantley, S. J., Paine, M. F., Oberlies, N. H. (2011). Isolation and identification of intestinal CYP3A inhibitors from cranberry (Vaccinium macrocarpon) using human intestinal microsomes. *Planta Med 77*, 265-270.

[163] Roth, M., Araya, J. J., Timmermann, B. N., Hagenbuch, B. (2011). Isolation of modulators of the liver specific organic anion-transporting polypeptides (OATPs) 1B1 and 1B3 from *Rollinia emarginata* Schlecht (Annonaceae). *J Pharmacol Exp Ther 339*, 624-632.

[164] Uesawa, Y. & Mohri, K. (2006). Effects of cranberry juice on nifedipine pharmacokinetics in rats. *J Pharm Pharmacol 58*, 1067-1072.

[165] Lilja, J. J., Backman, J. T., Neuvonen, P. J. (2007). Effects of daily ingestion of cranberry juice on the pharmacokinetics of warfarin, tizanidine, and midazolam-probes of CYP2C9, CYP1A2, and CYP3A4. *Clin Pharmacol Ther 81*, 833-839.

[166] Ngo, N., Yan, Z., Graf, T. N., Carrizosa, D. R., Kashuba, A. D., Dees, E. C., *et al*. (2009). Identification of a cranberry juice product that inhibits enteric CYP3A-mediated first-pass metabolism in humans. *Drug Metab Dispos 37*, 514-522.

[167] He, X. & Liu, R. H. (2006). Cranberry phytochemicals: Isolation, structure elucidation, and their antiproliferative and antioxidant activities. *J Agric Food Chem 54*, 7069-7074.

[168] Aggarwal, B. B. & Shishodia, S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol 71*, 1397-1421.

[169] Allouche, Y., Jiménez, A., Uceda, M., Aguilera, M. P., Gaforio, J. J., Beltrán, G. (2009). Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars. *J Agric Food Chem 57*, 3604-3610.

[170] Guinda, A., Rada, M., Delgado, T., Gutiérrez-Adánez, P., Castellano, J. M. (2010). Pentacyclic triterpenoids from olive fruit and leaf. *J Agric Food Chem 58*, 9685-9691.

[171] Chen, S. T., Dou, J., Temple, R., Agarwal, R., Wu, K. M., Walker, S. (2008). New therapies from old medicines. *Nat Biotechnol 26*, 1077-1083.

[172] Derendorf, H. & Hochhaus, G. (1995). Handbook of pharmacokinetic pharmacodynamic correlations (pharmacology and toxicology). Florida: CRC Press.

[173] Lilja, J. J., Kivistö, K. T., Neuvonen, P. J. (1999). Grapefruit juice increases serum concentrations of atorvastatin and has no effect on pravastatin. *Clin Pharmacol Ther 66*, 118-127.

[174] Fukazawa, I., Uchida, N., Uchida, E., Yasuhara H. (2004). Effects of grapefruit juice on pharmacokinetics of atorvastatin and pravastatin in Japanese. *Br J Clin Pharmacol 57*, 448-455.

[175] Ando, H., Tsuruoka, S., Yanagihara, H., Sugimoto, K., Miyata, M., Yamazoe, Y., *et al*. (2005). Effects of grapefruit juice on the pharmacokinetics of pitavastatin and atorvastatin. *Br J Clin Pharmacol 60*, 494-497.

[176] Cohen, E. E. W., Hartford, C., Maitland, M. L., Fox-Kay, K., Vogel, P., Wu, K., *et al*. (2009). Grapefruit juice significantly increases bioavailability of weekly rapamycin (sirolimus)-evidence of safety and activity in a phase 1 study. American Association for Cancer Research 100th Annual Meeting Abstract LB-135, Denver, CO, April 18-22 2009, American Association for Cancer Research.

[177] Liu, C., Shang, Y. F., Zhang, X. F., Zhang, X. G., Wang, B., Wu, Z., *et al*. (2009). Co-administration of grapefruit juice increases bioavailability of tacrolimus in liver transplant patients: a prospective study. *Eur J Clin Pharmacol 65*, 881-885.

[178] Ravi, P. R., Vats, R., Thakur, R., Srivani, S., Aditya, N. (2012). Effect of grapefruit juice and ritonavir on pharmacokinetics of lopinavir in wistar rats. *Phytother Res* DOI: 10.1002/ptr.4593.

[179] Asensi, M., Ortega, A., Mena, S., Feddi, F., Estrela, J. M. (2011). Natural polyphenols in cancer therapy. *Crit Rev Clin Lab Sci 48*, 197-216.

[180] Wu, C. P., Ohnuma, S., Ambudkar, S. V. (2011). Discovering natural product modulators to overcome multidrug resistance in cancer chemotherapy. *Curr Pharm Biotechnol 12*, 609-620.

[181] Gobburu, J. V. & Marroum, P. J. (2001). Utilisation of pharmacokinetic-pharmacodynamic modelling and simulation in regulatory decision-making. *Clin Pharmacokinet 40*, 883-892.

[182] Chien, J. Y., Friedrich, S., Heathman, M. A., de Alwis, D. P., Sinha, V. (2005). Pharmacokinetics/pharmacodynamics and the stages of drug development: role of modeling and simulation. *AAPS J 7*, E544-559.

[183] Miller, R., Ewy, W., Corrigan, B. W., Ouellet, D., Hermann, D., Kowalski, K. G., *et al*. (2005). How modeling and simulation have enhanced decision making in new drug development. *J Pharmacokinet Pharmacodyn 32*, 185-197.

[184] Holford, N., Ma, S. C., Ploeger, B. A. (2010). Clinical trial simulation: a review. *Clin Pharmacol Ther 88*, 166-182.

[185] Einolf, H. J. (2007). Comparison of different approaches to predict metabolic drug-drug interactions. *Xenobiotica 37*, 1257-1294.

[186] Pang, K. S. & Durk, M. R. (2010). Physiologically-based pharmacokinetic modeling for absorption, transport, metabolism and excretion. *J Pharmacokinet Pharmacodyn 37*, 591-615.

[187] Poulin, P., Jones, R. D., Jones, H. M., Gibson, C. R., Rowland, M., Chien, J. Y., *et al*. (2011). PHRMA CPCDC initiative on predictive models of human pharmacokinetics, part 5: Prediction of plasma concentration-time profiles in human by using the physiologically-based pharmacokinetic modeling approach. *J Pharm Sci* DOI: 10.1002/jps.22550.

[188] Rowland, M., Peck, C., Tucker, G. (2011). Physiologically-based pharmacokinetics in drug development and regulatory science. *Annu Rev Pharmacol Toxicol 10*, 45-73.

[189] Zhao P, Zhang L, Grillo JA, Liu Q, Bullock JM, Moon YJ, *et al*. (2011). Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clin Pharmacol Ther 89*, 259-267.

[190] Bouzom, F., Ball, K., Perdaems, N., Walther, B. (2012). Physiologically based pharmacokinetic (PBPK) modelling tools: how to fit with our needs? *Biopharm Drug Dispos 33*, 55-71.

[191] Galetin, A., Ito, K., Hallifax, D., Houston, J. B. (2005). CYP3A4 substrate selection and substitution in the prediction of potential drug-drug interactions. *J Pharmacol Exp Ther 314*, 180-190.

[192] Galetin, A., Burt, H., Gibbons, L., Houston, J. B. (2006). Prediction of time-dependent CYP3A4 drug-drug interactions: impact of enzyme degradation, parallel elimination pathways, and intestinal inhibition. *Drug Metab Dispos 34*, 166-175.

[193] Obach, R. S., Walsky, R. L., Venkatakrishnan, K., Houston, J. B., Tremaine, L. M. (2005). In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: qualitative relationships, quantitative predictions, and the rank-order approach. *Clin Pharmacol Ther 78*, 582-592.

[194] Obach, R. S., Walsky, R. L., Venkatakrishnan, K., Gaman, E. A., Houston, J. B., Tremaine, L. M. (2006). The utility of in vitro cytochrome P450 inhibition data in the prediction of drug-drug interactions. *J Pharmacol Exp Ther 316*, 336-348.

[195] Obach, R. S., Walsky, R. L., Venkatakrishnan, K. (2007). Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. *Drug Metab Dispos 35*, 246-255.

[196] Houston, J. B. & Galetin, A. (2008). Methods for predicting in vivo pharmacokinetics using data from in vitro assays. *Curr Drug Metab 9*, 940-951.

[197] Fahmi, O. A., Maurer, T. S., Kish, M., Cardenas, E., Boldt, S., Nettleton, D. (2008). A combined model for predicting CYP3A4 clinical net drug-drug interaction based on CYP3A4 inhibition, inactivation, and induction determined in vitro. *Drug Metab Dispos 36*, 1698-1708.

[198] Youdim, K. A., Zayed, A., Dickins, M., Phipps, A., Griffiths, M., Darekar, A., *et al*. (2008). Application of CYP3A4 in vitro data to predict clinical drug-drug interactions; predictions of compounds as objects of interaction. *Br J Clin Pharmacol 65*, 680-692.

[199] Obach, R. S. (2009). Predicting drug-drug interactions from in vitro drug metabolism data: challenges and recent advances. *Curr Opin Drug Discov Devel 12*, 81-89.

[200] Fahmi, O. A., Hurst, S., Plowchalk, D., Cook, J., Guo, F., Youdim, K., *et al*. (2009). Comparison of different algorithms for predicting clinical drug-drug interactions, based on the use of CYP3A4 in vitro data: predictions of compounds as precipitants of interaction. *Drug Metab Dispos 37*, 1658-1666.

[201] Fan, J., Chen, S., Chow, E. C., Pang, K. S. (2010). PBPK modeling of intestinal and liver enzymes and transporters in drug absorption and sequential metabolism. *Curr Drug Metab 11*, 743-761.

[202] Boulenc, X. & Barberan, O. (2011). Metabolic-based drug-drug interactions prediction, recent approaches for risk assessment along drug development. *Drug Metabol Drug Interact 26*, 147-168.

[203] Hisaka, A., Ohno, Y., Yamamoto, T., Suzuki, H. (2010). Theoretical considerations on quantitative prediction of drug-drug interactions. *Drug Metab Pharmacokinet 25*, 48-61.

[204] Bruyére, A., Declèves, X., Bouzom, F., Ball, K., Marques, C., Treton, X., *et al*. (2010). Effect of variations in the amounts of P-glycoprotein (ABCB1), BCRP (ABCG2) and CYP3A4 along the human small intestine on PBPK models for predicting intestinal first pass. *Mol Pharm 7*, 1596-1607.

[205] Guest, E. J., Rowland-Yeo, K., Rostami-Hodjegan, A., Tucker, G. T., Houston, J. B., Galetin, A. (2011). Assessment of algorithms for predicting drug-drug interactions via inhibition mechanisms: comparison of dynamic and static models. *Br J Clin Pharmacol 71*, 72-87.

[206] Shardlow, C. E., Generaux, G. T., MacLauchlin, C. C., Pons, N., Skordos, K. W., Bloomer, J. C. (2011). Utilizing drug-drug interaction prediction tools during drug development: enhanced decision making based on clinical risk. *Drug Metab Dispos 39*, 2076-2084.

[207] Galetin, A., Hinton, L. K., Burt, H., Obach, R. S., Houston, J. B. (2007). Maximal inhibition of intestinal first-pass metabolism as a pragmatic indicator of intestinal contribution to the drug-drug interactions for CYP3A4 cleared drugs. *Curr Drug Metab 8*, 685-693.

[208] Yang, J., Jamei, M., Yeo, K. R., Tucker, G. T., Rostami-Hodjegan, A. (2007). Prediction of intestinal first-pass drug metabolism. *Curr Drug Metab 8*, 676-684.

[209] Galetin, A., Gertz, M., Houston, J. B. (2008). Potential role of intestinal first-pass metabolism in the prediction of drug-drug interactions. *Expert Opin Drug Metab Toxicol 4*, 909-922.

[210] Galetin, A., Gertz, M., Houston, J. B. (2010). Contribution of intestinal cytochrome P450-mediated metabolism to drug-drug inhibition and induction interactions. *Drug Metab Pharmacokinet 25*, 28-47.

[211] Peters, S. A. (2008). Identification of intestinal loss of a drug through physiologically based pharmacokinetic simulation of plasma concentration-time profiles. *Clin Pharmacokinet 47*, 245-259.

[212] Tachibana, T., Kato, M., Takano, J., & Sugiyama, Y. (2010). Predicting drug-drug interactions involving the inhibition of intestinal CYP3A4 and P-glycoprotein. *Curr Drug Metab 11*, 762-777.

[213] Gertz, M., Harrison, A., Houston, J. B., & Galetin, A. (2010). Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos 38*, 1147-1158.

[214] Gertz, M., Houston, J. B., & Galetin A. (2011). Physiologically based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction. *Drug Metab Dispos 39*, 1633-1642.

[215] Tachibana, T., Kato, M., Sugiyama, Y. (2012). Prediction of nonlinear intestinal absorption of CYP3A4 and P-glycoprotein substrates from their in vitro Km values. *Pharm Res 29*, 651-668.

[216] Fenneteau, F., Poulin, P., Nekka, F. (2010). Physiologically based predictions of the impact of inhibition of intestinal and hepatic metabolism on human pharmacokinetics of CYP3A substrates. *J Pharm Sci 99*, 486-514.

[217] Lilja, J. J, Neuvonen, M., Neuvonen, P. J. (2004). Effects of regular consumption of grapefruit juice on the pharmacokinetics of simvastatin. *Br J Clin Pharmacol 58*, 56-60.

[218] van de Waterbeemd, H. Gifford, E. (2003). ADMET in silico modelling: towards prediction paradise? *Nat Rev Drug Discov 2*, 192-204.

[219] Benet L. Z., Cummins, C. L., Wu, C. Y. (2003). Transporter-enzyme interactions: implications for predicting drug-drug interactions from in vitro data. *Curr Drug Metab 4*, 393-398.

[220] Bolger, M. B., Lukacova, V., Woltosz, W. S. (2009). Simulations of the nonlinear dose dependence for substrates of influx and efflux transporters in the human intestine. *AAPS J 11*, 353-363.

[221] Gurley, B. J. (2010). Clinical pharmacology and dietary supplements: an evolving relationship. *Clin Pharmacol Ther 87*, 235-238.

CHAPTER 2

A MODIFIED GRAPEFRUIT JUICE ELIMINATES FURANOCOUMARINS AND POLYMETHOXYFLAVONES AS CANDIDATE MEDIATORS OF THE FEXOFENADINE-GRAPEFRUIT JUICE INTERACTION IN HEALTHY VOLUNTEERS

OVERVIEW

The grapefruit juice-fexofenadine interaction involves inhibition of intestinal organic anion transporting polypeptide (OATP)-mediated uptake. Only naringin has been shown clinically to inhibit intestinal OATP; other constituents have not been evaluated. Effects of a modified grapefruit juice devoid of furanocoumarins (~99%) and polymethoxyflavones (~90%) on fexofenadine disposition were compared to effects of the original juice. Extracts of both juices inhibited estrone 3-sulfate and fexofenadine uptake by similar extents in OATP-transfected cells (~50% and ~25%, respectively). Healthy volunteers (n=18) were administered fexofenadine (120 mg) with water, grapefruit juice, or modified grapefruit juice (240 ml) by randomized, three-way crossover design. Compared to water, both juices decreased fexofenadine geometric mean AUC and C_{max} by ~25% ($p \leq 0.008$ and $p \leq 0.011$, respectively), with no effect on terminal half-life ($p=0.11$). Similar effects by both juices on fexofenadine pharmacokinetics indicate furanocoumarins and polymethoxyflavones are not major mediators of the grapefruit juice-fexofenadine interaction.

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INTRODUCTION

The drug interaction liability of grapefruit juice (GFJ) and individual constituents has been studied extensively for more than 20 years [1, 2]. GFJ-drug interactions can manifest clinically as increased systemic drug exposure and subsequent adverse reactions due to mechanism-based inhibition of cytochrome P450 3A (CYP3A)-mediated metabolism in the intestine by GFJ [3, 4]. A class of constituents, furanocoumarins, was established as major mediators of this effect [4-6]. A different GFJ-type interaction was reported in 2002, when the non-sedating, minimally metabolized antihistamine fexofenadine was used as a probe substrate for examining the effect of GFJ on the efflux transporter, P-glycoprotein (P-gp), in the intestine [7]. Healthy volunteers administered fexofenadine with GFJ exhibited an unexpected mean 63% decrease in fexofenadine exposure. This seemingly paradoxical effect was observed consistently in four independent clinical studies reported over the subsequent five years [8-11]. The underlying mechanism was postulated to involve inhibition of fexofenadine active uptake in the intestine by organic anion transporting polypeptides (OATPs). Three clinical studies demonstrated that the size and flare of histamine-induced skin wheals were increased after administration of fexofenadine with GFJ or orange juice but not water [12]. Population pharmacokinetic analysis of the combined data from these studies and a bioequivalence study showed that the oral availability of fexofenadine was reduced by 36% [12]. Due to the potential for reduced therapeutic efficacy, the labeling of all fexofenadine products recommends taking the drug with water [12].

Constituents in GFJ have been identified as OATP inhibitors *in vitro* [11, 13] but the flavanone, naringin, is the only single constituent tested clinically [11]. Relative to water, GFJ and an aqueous solution of naringin at the same concentration as that in GFJ (~1,200 μmol/l) decreased fexofenadine mean exposure by 42 and 22%, respectively. A suspension of a particulate fraction of GFJ containing three one-hundredths the concentration of naringin (34 μmol/l) had no effect on fexofenadine exposure. The investigators concluded that naringin was a major causative ingredient inhibiting enteric OATP. However, the ~50% difference in fexofenadine exposure between GFJ and

naringin suggests other constituents contributed to the interaction.

The furanocoumarins, bergamottin and 6',7'-dihydroxybergamottin (DHB), and the polymethoxyflavones, tangeretin and nobiletin, have been reported to inhibit human enteric OATP activity *in vitro* [13]. Whether these observations translate to the clinic is not known. Based on the cumulative data, these two classes of GFJ constituents were evaluated as candidate OATP inhibitors by comparing the effect of a GFJ devoid of furanocoumarins and polymethoxyflavones (*i.e.*, modified GFJ, mGFJ) with that of the original GFJ on fexofenadine disposition in both OATP-transfected cells and healthy volunteers.

MATERIALS, SUBJECTS, AND METHODS

Materials and chemicals

[^{3}H]E1S ammonium salt (54.3 Ci/mmol) was purchased from Perkin Elmer (Waltham, MA). [^{3}H]Fexofenadine (6 Ci/mmol), originally a gift from GlaxoSmithKline (Research Triangle Park, NC) and custom synthesized by Amersham Life Sciences (Piscataway, NJ), was provided by Dr. Dhiren Thakker (Eshelman School of Pharmacy, Chapel Hill, NC). E1S potassium salt, verapamil (VER), bromosulfophthalein (BSP), BG, DHB, naringin, hesperidin, tangeretin, and nobiletin were purchased from Sigma-Aldrich (St. Louis, MO). Fexofenadine was obtained from Tocris Bioscience (Minneapolis, MN). Methanol, ethyl acetate, acetonitrile, calcium chloride, D-glucose, magnesium sulfate heptahydrate, potassium chloride, potassium phosphate monobasic, sodium chloride, sodium hydroxide, Triton X-100, 2-(*N*-morpholino)ethanesulfonic acid hydrate (MES), and scintillation cocktail were purchased from Fisher Scientific (Pittsburgh, PA). CV-1 (simian) origin SV40 virus (COS-1) and human embryonic kidney 293T/17 (HEK 293T/17) cells were obtained from American Type Culture Collection (Manassas, VA). Cell culture plates were purchased from Corning Life Sciences (Tewksbury, MA). Phosphate-buffered saline; fetal bovine serum; trypsin-EDTA; penicillin; streptomycin; Opti-MEM; DMEM containing 4.5 g/L D-glucose, 2 mM L-glutamine, and 110 mg/L sodium pyruvate; and Lipofectamine2000 were purchased from Invitrogen (Carlsbad, CA). X-tremeGENE 9 was purchased from Roche Applied Science (Indianapolis, IN). Human OATP1A2 (variant 1; accession number NM_134431.1) and OATP2B1 expression plasmids were obtained from Origene Technologies (Rockville, MD). Plasmids for mock-transfected cells pEYFP-C1 and pcDNA3.1/Hygro(+) were purchased from Clontech (Mountain View, CA) and Invitrogen, respectively. Madin-Darby canine kidney type II (MDCKII) parental cells and stably transfected MDCKII-OATP2B1 cells were obtained by material transfer agreement from Dr. Markus Grube (Ernst-Moritz-Arndt University, Greifswald, Germany).

Preparation of whole and modified grapefruit juice

A commercial GFJ concentrate for manufacture was obtained from a Florida processing

facility and prepared as described previously [6, 14]. Briefly, the concentrate was diluted with distilled water to yield single strength juice or was processed with a series of food-grade solvents and absorption resins to remove furanocoumarins (99%) and polymethoxyflavones (97%) but retain flavonones (70%). The original GFJ and modified GFJ (mGFJ) were pasteurized at 91°C for 8 s, cold-filled in containers sanitized with a 95°C water rinse, divided into 300-mL aliquots, and frozen to -20°C in 480-mL glass bottles. Due to the >5-y storage period between the most previous [14] and current clinical study, the GFJ and mGFJ aliquots were re-pooled, re-pasteurized at 71.7°C for 6 s, poured into sterilized bottles, and stored at -20°C until use. Representative compounds from the flavonone, furanocoumarin, and polymethoxyflavone classes were re-measured by HPLC as described previously [6, 14].

Preparation of whole and modified grapefruit juice extracts

Concentrated extracts of GFJ and mGFJ were prepared by adding 20 ml ethyl acetate to a 50-ml conical polypropylene tube containing 25 ml juice. Contents were shaken vigorously for 30 s and centrifuged (2500 *x g* for 30 min at 25 °C). The resulting organic layer was transferred to a 250-ml round-bottom glass flask. The extraction procedure was repeated twice, with each resultant organic layer combined in the flask. Organic layers were evaporated *in vacuo*. The residue was transferred to a 2-ml vial using methanol as a rinse and evaporated to dryness under air. The dried material was resuspended with 500 µl methanol, yielding a 200-fold concentrated extract of the starting juice volume (100 ml).

Transient transfection of OATP1A2 and OATP2B1 into COS-1 and HEK293T/17 cells

Human OATP1A2 and OATP2B1 expression plasmids were verified by DNA sequencing prior to use. COS-1 cells were maintained, seeded, and transfected transiently as described previously [15]. HEK293T/17 cells were cultured and maintained in DMEM supplemented with 10% fetal bovine serum at 37°C and 5% CO_2. Cells were seeded on Day 0 onto 100-mm dishes and transfected on Day 1 using the X-tremeGENE9 transfection reagent per manufacturer instructions. Transfection efficiency was assessed on Day 2 with fluorescence microscopy by estimating the percentage of cells

expressing EYFP (mock). On Day 3, transfected cells were trypsinized, seeded onto 24-well plates, and incubated for approximately 48 h before commencing the uptake assays.

Uptake and inhibition assays

On Day 4 or 5, cells were washed and preincubated for 30 min at 37°C in uptake buffer (pH 6) [15]. Buffer was replaced, and cells were incubated with a dosing solution (200 µl) consisting of radiolabeled (plus unlabeled) E1S (total: 5 µmol/l) or fexofenadine (total: 5 µmol/l) in the presence of vehicle (1% methanol), VER (250 µmol/l) and/or BSP (250 µmol/l), or diluted juice extract (1:200, 2:200). After 3 (E1S) or 30 (fexofenadine) min, cells were washed three times with ice-cold phosphate-buffered saline and lysed with either 0.1 N NaOH (COS-1 cells) or 1% Triton X-100 in phosphate-buffered saline (HEK293T/17 cells). Liquid scintillation cocktail (5 ml) was added to 200-µl aliquots of the cell lysates, and radioactivity was counted. Protein concentrations were determined with a BCA assay kit (Thermo Fisher Scientific, Waltham, MA). Uptake was linear over the selected times for each substrate (data not shown).

Clinical study protocol

The study protocol was reviewed and approved by the University of North Carolina Biomedical Institutional Review Board and Clinical and Translational Research Center (CTRC) Committee. All subjects provided written informed consent prior to participation. Healthy volunteers (9 women, 9 men) were enrolled. The median age (range) of the women and men was 30 (23-54) and 37 (23-60) y, respectively. Participants were self-identified as Caucasian (7 women, 5 men), African-American (2 women, 2 men), Asian (1 man), or Hispanic (1 man). Concomitant medications included oral/vaginal ring contraceptive therapy (2 Caucasian women), hydrochlorothiazide (1 Caucasian woman), low-dose aspirin (1 African-American man), multivitamin, calcium, folate, and ω-3 fatty acid supplements (3 women, 3 men). Prior to enrollment, each volunteer underwent a medical history, physical examination, and laboratory tests (*i.e.*, liver function tests, basic metabolic panel, complete blood count). All women underwent a serum pregnancy test. Subjects were instructed to abstain from all fruit juices for ≥7 d prior to and during the study and to abstain from alcohol and caffeine-

containing beverages the evening before each admission. Each subject was assigned randomly to 1 of 6 treatment sequences: ABC, CAB, BCA, CBA, BAC, or ACB (A = water, B = GFJ, C = mGFJ).

Eligible volunteers were admitted to the CTRC the evening prior to each of three study phases, which were separated by ≥10 d. All women underwent a repeat serum pregnancy test on the evening of each admission. Following an overnight fast beginning at midnight and placement of an indwelling venous catheter in an antecubital vein, each subject ingested two 60 mg fexofenadine tablets (Prasco Laboratories, Mason, OH) with 240 ml water, GFJ, or mGFJ. Blood (7 ml) was collected by venipuncture into EDTA-containing tubes (Becton-Dickinson, Franklin Lakes, NJ) before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 h after fexofenadine administration. Subjects continued to fast until after the 4-h blood collection, after which meals and snacks, devoid of fruit juices and caffeinated beverages, were provided. Vital signs were recorded at baseline and monitored periodically. Subjects were discharged after the 12-h blood collection and returned for outpatient blood draws at 24, 36, 48, and 72 h post-fexofenadine administration. Plasma was separated from blood cells by centrifugation within 1 h of collection, transferred into cryovials, and stored at -80°C pending analysis for fexofenadine.

Analysis of plasma for fexofenadine

Plasma collections were processed by transferring 50 µl to a 96-well plate insert and precipitating proteins with 150 µl of methanol containing fexofenadine-d_6 (1 nmol/l) as internal standard (Toronto Research Chemicals Inc., Toronto, ON, Canada). The mixtures were vortex-mixed for 5 min and centrifuged (3000 *x g* for 10 min at 4°C). Calibration solutions (0.0012-7.2 µmol/l) and quality controls (1, 0.5, 0.1, 0.05, 0.005 µmol/l) were prepared similarly using fexofenadine and multiple-donor pooled plasma (Biological Specialty Corporation, Colmar, PA). Plasma was analyzed for fexofenadine by HPLC-tandem mass spectrometry using an API 4000 triple quadrupole with TurboIonSpray interface (Applied Biosystems/MDS Sciex, Concord, ON, Canada) as described [16]. Briefly, 5 µl were injected, and fexofenadine and fexofenadine-d_6 were eluted from an Aquasil C18 column (2.1 x 50 mm, particle diameter = 5 µm; Thermo Fisher Scientific, Waltham, MA) using a

mobile phase gradient (A: 0.1% formic acid in water; B: 0.1% formic acid in methanol) at a flow rate of 0.75 ml/min. The mass spectrometer was operated in positive-ion mode. Multiple reaction monitoring was used to detect fexofenadine (502 → 466 *m*/*z*) and fexofenadine-d_6 (508 → 472 *m*/*z*). The lower limit of quantification was 0.0012 μmol/l; inter- and intra-day coefficients of variation were 12 and <15%, respectively, for the quality controls.

Data analysis

OATP1A2- and OATP2B1-mediated uptake, in the absence or presence of inhibitor, was normalized for protein content. Net uptake was determined by subtracting the uptake in mock-transfected cells from that in OATP1A2- and OATP2B1-expressing cells incubated under parallel conditions.

Fexofenadine pharmacokinetics were evaluated by non-compartmental methods using WinNonlin (v 5.2, Pharsight Corp., Mountain View, CA). The terminal elimination rate constant (λ_z) was estimated by log-linear regression of at least the last three data points in the terminal phase of the plasma concentration-time profile. The terminal half-life ($t_{½}$) was calculated as $0.693/\lambda_z$. The maximum concentration (C_{max}), time to reach C_{max} (t_{max}), and the last measurable concentration (C_{last}) were determined visually from the concentration-time profile. $AUC_{0\text{-}last}$ was calculated using the trapezoidal rule with linear up/log down interpolation. AUC from zero to infinite time ($AUC_{0\text{-}\infty}$) was calculated as the sum of AUC_{last} and C_{last}/λ_z. Apparent oral clearance (Cl/F) was calculated as $Dose/AUC_{0\text{-}\infty}$. Below limit of quantification concentrations were excluded from data analysis.

Statistical analysis

Statistical analysis employed SigmaPlot (v 11, Systat Software, Inc., San Jose, CA). *In vitro* data are presented as means ± SD of triplicate incubations. Two-way analysis of variance (ANOVA) followed by Tukey's test was used to test for differences between vehicle and inhibitor treatments. Student's unpaired *t*-test was used to test for differences between mock- and OATP-transfected cells. A p-value < 0.05 was considered statistically significant.

Pharmacokinetic outcomes ($t_{½}$, C_{max}, $AUC_{0\text{-}\infty}$, Cl/F) are reported as geometric means with

coefficients of variation (CV%). Medians and ranges are reported for t_{max}. The Wilcoxon signed-rank test was used to compare t_{max}. Pharmacokinetic outcomes, expressed as the ratio of GFJ to water or GFJ to mGFJ, are reported as geometric means with 90% confidence intervals. Comparisons using one-tailed paired Student's *t*-tests with Bonferroni correction (*i.e.*, 0.05/2) were used to detect differences between water and GFJ and between GFJ and mGFJ treatments. A p-value ≤ 0.025 was considered statistically significant. Based on the method proposed by Lauzon and Caffo [17], for the primary pharmacokinetic measure of interest, AUC, a minimum of 18 subjects who completed all treatment phases was deemed adequate for this study, assuming a within-subject CV of 23% for fexofenadine determined from a previous study [11]. The randomization scheme was generated using SAS (v 9.2, SAS Institute Inc., Cary, NC).

RESULTS

Representative furanocoumarin, polymethoxyflavone, and flavanone concentrations in grapefruit juice and modified grapefruit juice

Due to the >5-y lapse since last use [6, 14], GFJ and mGFJ were re-analyzed for representative compounds from each of three phytochemical classes shown to inhibit intestinal OATP activity *in vitro* (Table 2.1). Relative to GFJ, the representative furanocoumarins DHB and bergamottin in mGFJ were reduced by >99% and 95%, respectively. The representative polymethoxyflavones nobiletin and tangeretin were reduced by 95 and 73%, respectively. The representative flavanones naringin, narirutin, and hesperidin were reduced by ~30, 32, and 44%, respectively. Mean (±SD) aggregate representative furanocoumarins, polymethoxyflavones, and flavanones measured in GFJ (48 ± 0.3, 0.48 ± 0.03, and 1,064 ± 10.4 μmol/l, respectively) and in mGFJ (0.6 ± 0.09, 0.05 ± 0.02, and 727 ± 14 μmol/l, respectively) were consistent with those measured initially (GFJ: 59 ± 2.1, 0.71 ± 0.03, and 1,012 ± 24.1 μmol/l; mGFJ: 0.3 ± 0.006, 0.03 ± 0.007, and 787 ± 68.6 μmol/l) [6, 14]. The net loss of ≤3% indicated negligible degradation over the >5-y storage period.

Effects of grapefruit juice and modified grapefruit juice extracts on estrone 3-sulfate and fexofenadine uptake in OATP-transfected cells

Ethyl acetate extracts of GFJ and mGFJ were evaluated as inhibitors of OATP1A2- and OATP2B1-mediated uptake in COS-1 and HEK 293T/17 cells using the probe substrate estrone 3-sulfate (E1S) to assess functional activity of the juices prior to clinical study conduct. Both juice extracts inhibited E1S uptake in COS-1 cells by at least 50% relative to vehicle (Figure 2.1A and 2.1B). Single-strength GFJ and mGFJ extracts inhibited OATP1A2-mediated uptake by 70 and 80%, respectively; double-strength extracts inhibited uptake by 84 and 50%, respectively (Figure 2.1A). Single-strength GFJ and mGFJ extracts inhibited OATP2B1-mediated uptake by 78 and 61%, respectively; double-strength extracts inhibited uptake by 52 and 74%, respectively (Figure 2.1B). Both juice extracts inhibited E1S uptake in HEK293T/17 cells by at least 45% relative to vehicle

(Figure 2.1C and 2.1D). Single-strength GFJ and mGFJ extracts inhibited OATP1A2-mediated uptake by 90 and 62%, respectively; double-strength extracts inhibited uptake by 80 and 74%, respectively (Figure 2.1C). Single-strength GFJ and mGFJ extracts inhibited OATP2B1-mediated uptake by 74 and 59%, respectively; double-strength extracts inhibited uptake by 60 and 45%, respectively (Figure 2.1D). Bromosulfophthalein (BSP), a non-specific OATP inhibitor, inhibited uptake by >80% in all cell systems.

COS-1 cells transiently overexpressing OATP1A2 were employed initially to gain mechanistic insight into inhibition of fexofenadine uptake by GFJ and mGFJ. Due to high uptake activity in mock (EYFP)-transfected cells with the juice extracts and BSP (Figure 2.2A), OATP1A2 was expressed transiently in HEK293T/17 cells, in which uptake in mock-transfected cells was more consistent (Figure 2.2B). Relative to vehicle-treated OATP1A2-transfected cells, both single- and double-strength GFJ extract inhibited fexofenadine uptake by ~27%. Both single- and double-strength mGFJ extract inhibited uptake by ~40%. Because BSP did not inhibit fexofenadine uptake, verapamil (VER) was used as an alternate OATP1A2 inhibitor, inhibiting by ~50%.

OATP2B1-mediated uptake of fexofenadine was evaluated in transiently transfected COS-1 and stably transfected MDCKII cells (Figure 2.3). Due to the possibility of cell line-dependent expression/uptake, OATP2B1 was expressed in HEK293T/17 cells. Fexofenadine uptake was not observed with any cell line.

Effects of grapefruit juice and modified grapefruit juice on fexofenadine pharmacokinetics in healthy volunteers

The effects of GFJ and mGFJ on fexofenadine pharmacokinetics were compared in 18 healthy participants. None of the participants withdrew from the study. Each treatment was well tolerated by all participants. No side effects were reported.

The geometric mean concentration-time profiles of fexofenadine in the presence of GFJ and mGFJ were nearly superimposable (Figure 2.4). The percentage of fexofenadine AUC extrapolated to infinite time ($AUC_{0-\infty}$) was <10% in all subjects and in all phases. Relative to water, GFJ and mGFJ

decreased geometric mean $AUC_{0-\infty}$ by ~25% (Table 2.2). The geometric mean $AUC_{0-\infty}$s were similar between GFJ and mGFJ (Figure 2.5). Both juices increased geometric mean Cl/F by ~33% (Table 2.2). Relative to water, both juices decreased geometric mean C_{max} by ~23% (Table 2.2). The geometric mean terminal $t_{½}$ and median t_{max} did not differ between treatments (Table 2.2).

DISCUSSION

Since discovery of the fruit juice-OATP interaction, candidate inhibitors of OATP isoforms such as OATP1A2 and OATP2B1 have been proposed and evaluated for effects on both hepatic and intestinal drug uptake [20-22]. The GFJ and mGFJ tested in the current work were compared previously in two clinical studies involving the CYP3A substrate felodipine [6] and the dual CYP3A/P-gp substrate cyclosporine [14]. The felodipine study established furanocoumarins, in aggregate, as major inhibitors of enteric CYP3A. The cyclosporine study further substantiated furanocoumarins as major inhibitors of enteric CYP3A, and likely P-gp; in addition, polymethoxyflavones were ruled out as inhibitors of enteric P-gp. Based on these observations, this unique GFJ-mGFJ combination permitted both *in vitro* and clinical evaluation of the collective impact of furanocoumarins and polymethoxyflavones on the absorption of a growing class of drugs whose enteric uptake depends on OATPs. Because no "clean" OATP substrates suitable for human use have been identified, coupled with prior knowledge of the GFJ-fexofenadine interaction [7-11], fexofenadine was selected as a third prototypic probe substrate to test with this GFJ and mGFJ.

Before clinical testing, mGFJ was characterized further by comparing to the original GFJ as an enteric OATP inhibitor in OATP1A2- and OATP2B1-overexpressing cell systems using the probe substrate E1S. Compared to vehicle, both juice extracts inhibited both OATP isoforms by >50% in COS-1 and HEK293T/17 cells. Inhibition of E1S uptake by both extracts also was observed in stably transfected MDCKII-OATP2B1 cells (data not shown) and is consistent with previous observations with stably transfected HEK293 cells and dilutions of whole GFJ [3]. The similar extents of OATP inhibition by the two extracts in HEK293T/17 cells predicted that systemic fexofenadine exposure in the clinical study would be comparable between juice treatments.

As anticipated, relative to water, GFJ decreased the geometric mean systemic exposure (AUC, C_{max}) to fexofenadine. This observation, coupled with the lack of an effect on geometric mean terminal half-life, was consistent with inhibition of uptake in the intestine by GFJ. The decrease in AUC (~25%) was near the low end compared to previous GFJ-fexofenadine studies (31 to 67%) [7-

11], which could be attributed to the highly variable concentrations of bioactive ingredients among commercial brands and batches of GFJ [23, 24]. The modest decrease in AUC reflects considerable interindividual variability in magnitude of effect, which ranged from -68 to +57% among the 18 subjects. This variability could be attributed in part to polymorphisms in the genes that encode for OATP (*SLCO*), as well as P-gp (*MDR1*); however, genotyping for relevant polymorphisms was beyond the scope of this study.

As predicted by the enteric OATP-transfected cells using E1S as the probe substrate, mGFJ decreased geometric mean systemic fexofenadine exposure to a similar extent as GFJ. The nearly identical effects by both juices on fexofenadine pharmacokinetics indicated furanocoumarins and polymethoxyflavones are not major mediators of the GFJ-fexofenadine interaction. Elimination of furanocoumarins as major *in vivo* inhibitors of enteric OATPs is consistent with the single clinical study involving 12 healthy volunteers administered fexofenadine (120 mg) and an aqueous suspension (300 ml) of a particulate fraction of GFJ, which contained mostly furanocoumarins (measurements not provided) and a relatively trivial concentration (34 μmol/l) of the flavanone naringin [11]. Fexofenadine AUC in the presence of the particulate fraction was similar to that in the presence of water. Naringin also was tested in the same clinical study [11] at the same concentration as that in whole GFJ (~1,200 μmol/l); the single constituent explained only half of the reduction in fexofenadine exposure caused by GFJ, indicating other constituents, possibly other flavanones (*e.g.*, narirutin, hesperidin), contribute to the interaction.

As aforementioned, fexofenadine is not a clean OATP substrate. Numerous investigators have shown that fexofenadine also is a P-gp substrate [25-30]. Accordingly, the diluted extracts of the clinically administered juices were tested as inhibitors of fexofenadine uptake in various OATP1A2- or OATP2B-transfected kidney-derived cell lines. Initial optimization studies with COS-1 cells demonstrated that fexofenadine was a weak substrate for OATP1A2. Low fexofenadine uptake by OATP1A2, coupled with high background activity, rendered difficulty in ascertaining the effects of the juice extracts. However, unlike COS-1 cells, the juice extracts did not significantly alter

fexofenadine uptake in mock-transfected HEK293T/17 cells. Due to higher fexofenadine uptake activity in OATP1A2-transfected HEK293T/17 cells, comparable inhibition by both juice extracts was demonstrated. In addition to P-gp and OATP, apical and basolateral efflux mediated by multidrug resistant protein (MRP) 2 and MRP3, respectively, have been shown to contribute to fexofenadine transport *in vitro* [31]. Studies with transiently transfected COS-1 and HEK293T/17 cells, as well as stably transfected MDCKII cells, demonstrated that fexofenadine is not a substrate for OATP2B1. Conflicting *in vitro* data have been reported regarding OATP2B1-mediated transport of fexofenadine. Fexofenadine uptake in transiently transfected HeLa and stably transfected HEK293 cells either was not evident or was weak [10, 32, 33]. However, an independently generated MDCKII-OATP2B1 cell line showed an ~3-fold difference in fexofenadine uptake compared to mock-transfected cells [31]. Fexofenadine has been shown to be a substrate of OATP2B1 in a *Xenopus laevis* oocyte system [34]. The discrepancy between *in vitro* experiments with fexofenadine may be attributed to multiple binding sites on OATP2B1 with different affinities, but to date, substrate-dependent binding has been demonstrated only with transfected oocytes [35].

In summary, current *in vitro* and clinical observations collectively indicate that furanocoumarins and polymethoxyflavones are not major mediators of the GFJ-fexofenadine interaction. Flavanones are likely candidate inhibitors of enteric OATPs *in vivo*. A bioactivity-guided fractionation approach similar to that used to identify intestinal CYP3A inhibitors in cranberry [36] and hepatic OATP inhibitors in *Rollinia emarginata* [37] can be used to identify specific causative ingredients. Identification of such OATP inhibitors would permit evaluation and prediction of the interaction liability of GFJ and other foods containing the same compounds with other existing and potential OATP substrates, including some fluoroquinolones, beta-blockers, and statins [38]. Finally, confirmation of causative mediators could impact food manufacturing practices, leading to advances designed to minimize interference with drugs, including removal of compounds *via* chemical processing or plant gene engineering [39-40].

Table 2.1. Concentrations of representative furanocoumarins, polymethoxyflavones, and flavanones in grapefruit juice (GFJ) and modified GFJ (mGFJ)

	Mean Concentration (µmol/l) (SD)	
Constituent	GFJ	mGFJ
Furanocoumarins		
DHB	38.8 (0.27)	0.13 (0.01)
Bergamottin	9.07 (0.04)	0.50 (0.08)
Polymethoxyflavones		
Nobiletin	0.37 (0.02)	0.02 (0.005)
Tangeretin	0.11 (0.01)	0.03 (0.01)
Flavanones		
Naringin	770 (5.24)	531 (7.86)
Narirutin	271 (3.98)	183 (5.43)
Hesperidin	24.6 (1.16)	14.7 (0.57)

DHB, 6',7'-dihydroxybergamottin

Table 2.2. Pharmacokinetics of fexofenadine (120 mg) in 18 healthy volunteers after administration with 240 ml of water, grapefruit juice (GFJ), or modified GFJ (mGFJ)

Outcome	Geometric Mean (CV%)			Geometric Mean Ratio (90% CI)	
	Water	GFJ	mGFJ	GFJ/Water	GFJ/mGFJ
$AUC_{0-\infty}$ (µmol/l ·h)	4.22 (40)	3.22[a] (33.5)	3.15 (28.6)	0.76 (0.3-1.4)	0.98 (0.4-1.5)
Cl/F (l/h)	52.8 (40)	69.3[b] (33.5)	70.9 (29.6)	1.31 (0.7-1.92)	1.02 (0.45-1.6)
C_{max} (µmol/l)	0.57 (52.2)	0.45[c] (43.7)	0.44 (32.4)	0.78 (0.17-1.39)	0.97 (0.35-1.6)
$t_{½}$ (h)	11.9 (36.5)	10.3[d] (37.6)	10.3 (29.7)	0.86 (0.28-1.45)	1.01 (0.43-1.58)
t_{max} (h) [median (range)]	2.69 (1-6)	3.23[e] (1.5-5)	3.54 (2-6)		

$AUC_{0-\infty}$, area the curve from time zero to infinity; Cl/F, apparent oral clearance; C_{max}, maximum concentration; $t_{½}$, terminal half-life; t_{max}, time to C_{max}

Statistical comparisons for all outcomes except t_{max} were made between water and GFJ using a one-tailed paired Student's *t*-test with Bonferroni correction.

[a] $p = 0.008$

[b] $p = 0.023$

[c] $p = 0.011$

[d] $p = 0.11$

[e] $p = 0.135$ (Wilcoxon signed-rank test)

FIGURE LEGENDS

Figure 2.1. Inhibitory effects of grapefruit juice extracts (GFJ, mGFJ) on estrone 3-sulfate (E1S) uptake in COS-1 (A, B) and HEK293T/17 (C, D) cells overexpressing human OATP1A2 (A, C) or OATP2B1 (B, D). Cells were transiently transfected with human OATP1A2 (black bars), OATP2B1 (black bars), or mock (EYFP) plasmids (gray bars). Cells were incubated for 3 min in pH 6.0 buffer at 37ºC with 0.05 μmol/l [^{3}H]E1S in the presence of vehicle (1% methanol) or GFJ and mGFJ extracts at single-strength (1X) or double-strength (2X). Bromosulfophthalein (BSP) (250 μmol/l) was used as a positive control inhibitor. E1S mean net uptake by OATP1A2 in the presence of vehicle was 17.5 ± 3.2 and 17.3 ± 1.13 pmol/mg protein/3 min, for COS-1 and HEK293T/17 cells, respectively. E1S mean net uptake by OATP2B1 in the presence of vehicle was 42.7 ± 3.0 and 17.7 ± 3.5 pmol/mg protein/3 min for COS-1 and HEK293T/17 cells, respectively. Bars and error bars denote means and SDs, respectively, of triplicate incubations. *$p < 0.05$ versus vehicle (Two-way ANOVA followed by Tukey's test), $^{\#}p < 0.05$ versus GFJ 1X (Two-way ANOVA followed by Tukey's test), $^{\dagger}p < 0.05$ versus GFJ 2X (Two-way ANOVA followed by Tukey's test), $^{\ddagger}p < 0.05$ versus mGFJ 1X (Two-way ANOVA followed by Tukey's test).

Figure 2.2. Inhibition of fexofenadine uptake by grapefruit juice extracts (GFJ, mGFJ) in OATP1A2-transfected cells. COS-1 (A) and HEK293T/17 (B) cells were transiently transfected with human OATP1A2 (black bars) or mock (EYFP) plasmids (gray bars). Cells were incubated for 30 min in pH 6.0 buffer at 37ºC with 0.5 μmol/l [^{3}H]fexofenadine in the presence of vehicle (1% methanol) or GFJ and mGFJ extracts at single-strength (1X) or double-strength (2X). Bromosulfophthalein (BSP) and verapamil (VER) (250 μmol/l) were used as positive control inhibitors. Fexofenadine mean net uptake by OATP1A2 in the absence of juice extracts was 12.8 ± 1.1 and 39.4 ± 4.8 pmol/mg protein/30 min, for COS-1 and HEK293T/17 cells, respectively. Bars and error bars denote means and SDs, respectively, of triplicate incubations. *$p < 0.05$ versus mock-transfected cells (Student's unpaired *t*-test). $^{\#}p < 0.05$ versus vehicle-treated OATP1A2-transfected cells (Two-way ANOVA followed by Tukey's test).

Figure 2.3. Fexofenadine uptake in OATP2B1-transfected cells. Transiently transfected COS-1 cells and HEK293T/17 cells and stably transfected MDCKII cells were incubated for 30 min in pH 6.0 buffer at 37ºC with 0.5 μmol/l [^{3}H]fexofenadine. Estrone 3-sulfate (E1S) was used as a positive control; fold-difference between control and transfected cells was 4, 5, and 20 for COS-1, MDCKII, and HEK293T/17 cells, respectively. Black bars represent OATP-mediated uptake. Gray bars represent mock-transfected (COS-1, HEK293T/17) or parental cell uptake (MDCKII). Bars and error bars denote means and SDs, respectively, of triplicate incubations.

Figure 2.4. Geometric mean plasma fexofenadine concentration-time profile following coadministration with 240 ml of water, grapefuit juice (GFJ), or modified GFJ (mGFJ) for 18 healthy volunteers. Symbols and error bars denote geometric means and upper limits of the 90% confidence interval, respectively. Inset depicts the 0 to 72 h profile. The 2-h time point of the water phase represents the geometric mean of 17 subjects. Open circles, closed squares, and closed triangles denote water, GFJ, and mGFJ, respectively.

Figure 2.5. Fexofenadine AUC changes in 18 healthy volunteers administered fexofenadine with 240 ml water, GFJ, and mGFJ. Open symbols and solid lines denote individual values. Closed symbols and dashed lines denote geometric means.

Figure 2.1

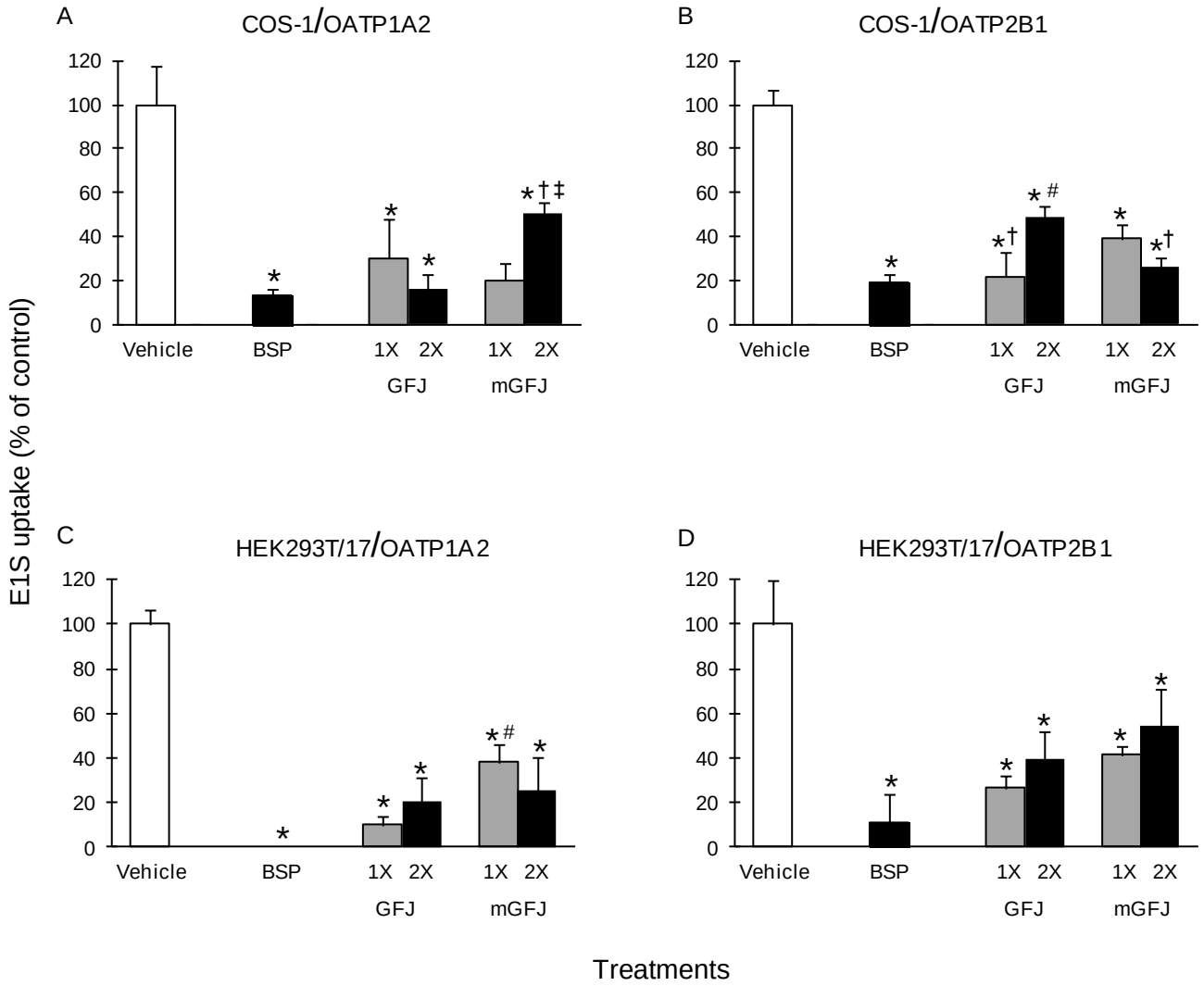

A
COS-1/OATP1A2
B
COS-1/OATP2B1
C
HEK293T/17/OATP1A2
D
HEK293T/17/OATP2B1
E1S uptake (% of control)
120
100
80
60
40
20
0
Vehicle
BSP
1X
2X
1X
2X
GFJ
mGFJ
Treatments

Figure 2.2

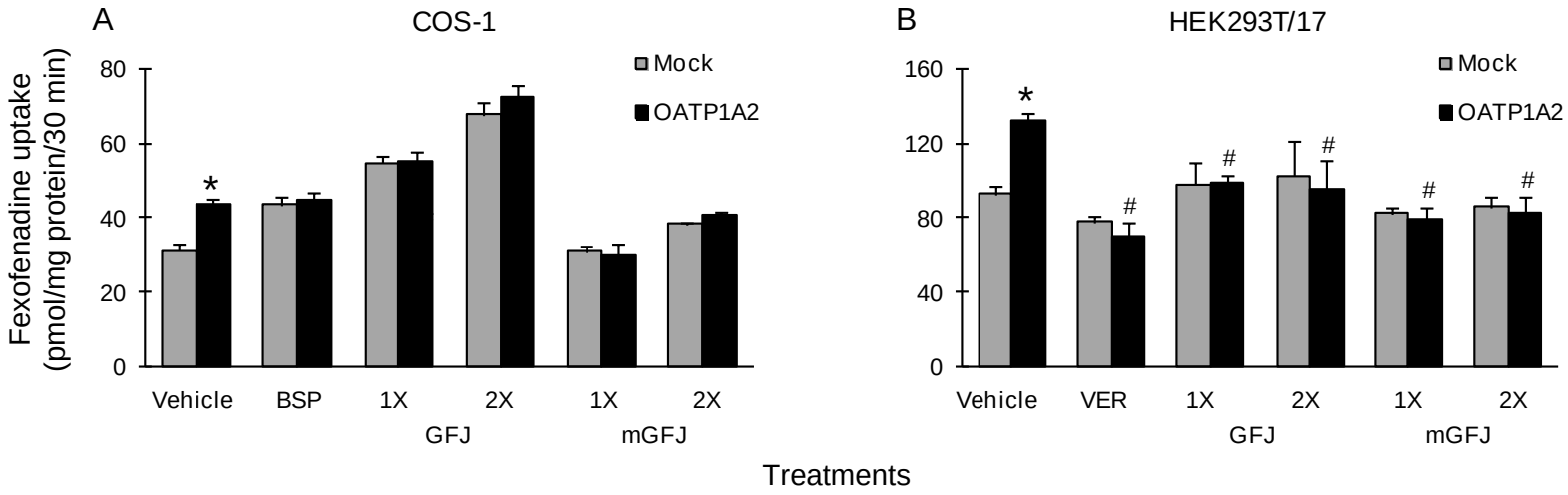

A
COS-1
Mock
OATP1A2
Fexofenadine uptake (pmol/mg protein/30 min)
80
60
40
20
0
*
Vehicle
BSP
1X
2X
1X
2X
GFJ
mGFJ
B
HEK293T/17
Mock
OATP1A2
160
120
80
40
0
*
#
#
#
#
Vehicle
VER
1X
2X
1X
2X
GFJ
mGFJ
Treatments

Figure 2.3

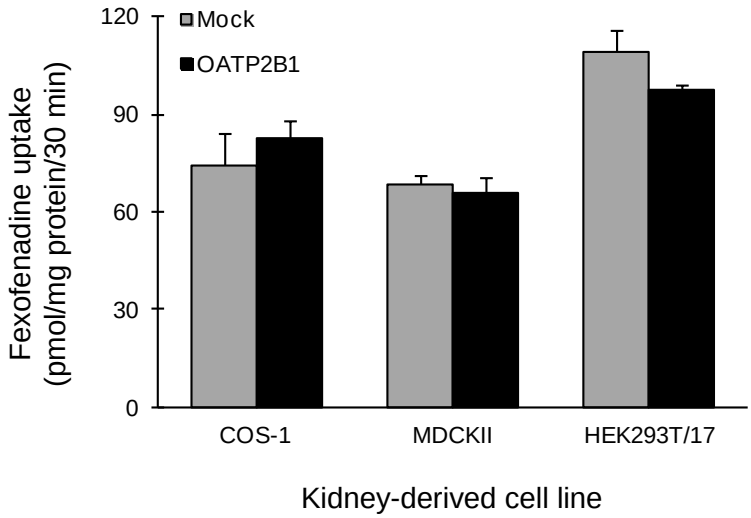

120
90
60
30
0
Mock
OATP2B1
Fexofenadine uptake
(pmol/mg protein/30 min)
COS-1
MDCKII
HEK293T/17
Kidney-derived cell line

Figure 2.4

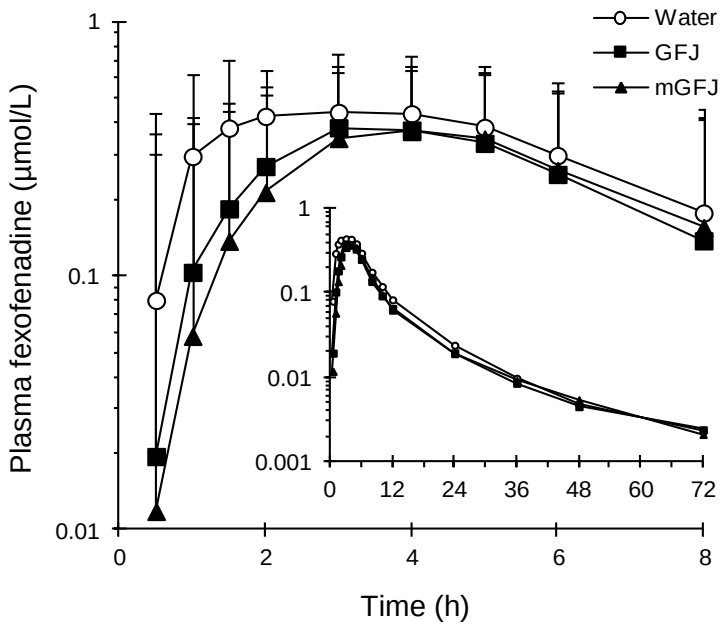
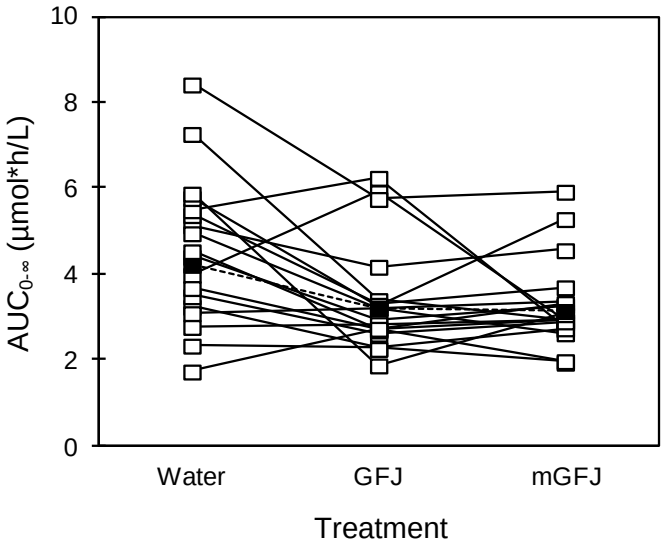

Water
GFJ
mGFJ
Plasma fexofenadine (µmol/L)
Time (h)
1
0.1
0.01
0
2
4
6
8
0.001
12
24
36
48
60
72

Figure 2.5



REFERENCES

[1] Mertens-Talcott SU, Zadezensky I, De Castro WV, Derendorf H, Butterweck V. Grapefruit-drug interactions: can interactions with drugs be avoided? *J Clin Pharmacol* 2006;46(12):1390-1416.

[2] Hanley MJ, Cancalon P, Widmer WW, Greenblatt DJ. The effect of grapefruit juice on drug disposition. *Expert Opin Drug Metab Toxicol* 2011;7(3):267-286.

[3] Lown KS, Bailey DG, Fontana RJ, et al. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* 1997;99(10):2545-2553.

[4] Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME, et al. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. *Drug Metab Dispos* 1997;25(11):1228-1233.

[5] Guo LQ, Fukuda K, Ohta T, Yamazoe Y. Role of furanocoumarin derivatives on grapefruit juice mediated inhibition of human CYP3A activity. *Drug Metab Dispos* 2000;28(7):766-771.

[6] Paine MF, Widmer WW, Hart HL, et al. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. *Am J Clin Nutr* 2006;83(5):1097-1105.

[7] Dresser GK, Bailey DG, Leake BF, et al. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* 2002;71(1):11-20.

[8] Banfield C, Gupta S, Marino M, Lim J, Affrime M. Grapefruit juice reduces the oral bioavailability of fexofenadine but not desloratadine. *Clin Pharmacokinet* 2002;41(4):311-318.

[9] Dresser GK, Kim RB, Bailey DG. Effect of grapefruit juice volume on the reduction of fexofenadine bioavailability: possible role of organic anion transporting polypeptides. *Clin Pharmacol Ther* 2005;77(3):170-177.

[10] Glaeser H, Bailey DG, Dresser GK, et al. Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin Pharmacol Ther* 2007;81(3):362-370.

[11] Bailey DG, Dresser GK, Leake BF, Kim RB. Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. *Clin Pharmacol Ther* 2007;81(4):495-502.

[12] Allegra [package insert]. Bridgewater, NJ: sanofi-aventis; 2008 Dec.

[13] Satoh H, Yamashita F, Tsujimoto M, et al. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos* 2005;33:518-523.

[14] Paine MF, Widmer WW, Pusek SN, et al. Further characterization of a furanocoumarin-free grapefruit juice on drug disposition: studies with cyclosporine. *Am J Clin Nutr* 2008;87(4):863-871.

[15] Lan T, Rao A, Haywood J, et al. Interaction of macrolide antibiotics with intestinally expressed human and rat organic anion-transporting polypeptides. *Drug Metab Dispos* 2009;37(12):2375-2382.

[16] Zhao R, Kalvass JC, Yanni SB, Bridges AS, Pollack GM. Fexofenadine brain exposure and the influence of blood-brain barrier P-glycoprotein after fexofenadine and terfenadine administration. *Drug Metab Dispos* 2009;37(3):529-535.

[17] Lauzon C, Caffo B. Easy multiplicity control in equivalence testing using two one-sided tests. *Am Stat* 2009;63(2):147-154.

[18] Greenblatt DJ. Analysis of drug interactions involving fruit beverages and organic anion-transporting polypeptides. *J Clin Pharmacol* 2009;49(12):1403-1407.

[19] Bailey DG. Fruit juice inhibition of uptake transport: a new type of food-drug interaction. *Br J Clin Pharmacol* 2010;70(5):645-655.

[20] Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* 2009;158(3):693-705.

[21] Hagenbuch B, Gui C. Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica* 2008;38(7-8):778-801.

[22] König J. Uptake transporters of the human OATP family: molecular characteristics, substrates, role in drug-drug interactions, and functional consequences of polymorphisms. *Handb Exp Pharmacol* 2011;(201):1-28.

[23] Ho PC, Saville DJ, Coville PF, Wanwimolruk S. Content of CYP3A4 inhibitors, naringin, naringenin and bergapten in grapefruit and grapefruit juice products. *Pharm Acta Helv* 2000;74(4):379-385.

[24] De Castro WV, Mertens-Talcott S, Rubner A, Butterweck V, Derendorf H. Variation of flavonoids and furanocoumarins in grapefruit juices: a potential source of variability in grapefruit juice-drug interaction studies. *J Agric Food Chem* 2006;54(1):249-255.

[25] Cvetkovic M, Leake B, Fromm MF, Wilkinson GR, Kim RB. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos* 1999;27(8):866-871.

[26] Dagenais C, Ducharme J, Pollack GM. Uptake and efflux of the peptidic delta-opioid receptor agonist. *Neurosci Lett* 2001;301(3):155-158.

[27] Perloff MD, von Moltke LL, Greenblatt DJ. Fexofenadine transport in Caco-2 cells: inhibition with verapamil and ritonavir. *J Clin Pharmacol* 2002;42(11):1269-1274.

[28] Tannergren C, Petri N, Knutson L, Hedeland M, Bondesson U, Lennernäs H. Multiple transport mechanisms involved in the intestinal absorption and first-pass extraction of fexofenadine. *Clin Pharmacol Ther* 2003;74(5):423-436.

[29] Petri N, Tannergren C, Rungstad D, Lennernäs H. Transport characteristics of fexofenadine in the Caco-2 cell model. *Pharm Res* 2004;21(8):1398-1404.

[30] Tahara H, Kusuhara H, Fuse E, Sugiyama Y. P-glycoprotein plays a major role in the efflux of fexofenadine in the small intestine and blood-brain barrier, but only a limited role in its biliary excretion. *Drug Metab Dispos* 2005;33(7):963-968.

[31] Ming X, Knight BM, Thakker DR. Vectorial transport of fexofenadine across Caco-2 cells: involvement of apical uptake and basolateral efflux transporters. *Mol Pharm* 2011;8(5):1677-1686.

[32] Shimizu M, Fuse K, Okudaira K, et al. Contribution of OATP (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans. *Drug Metab Dispos* 2005;33(10):1477-1481.

[33] Nozawa T, Imai K, Nezu J, Tsuji A, Tamai I. Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J Pharmacol Exp Ther* 2004;308(2):438-445.

[34] Imanaga J, Kotegawa T, Imai H, et al. The effects of the SLCO2B1 c.1457C>T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenet Genomics* 2011;21(2):84-93.

[35] Shirasaka Y, Mori T, Shichiri M, Nakanishi T, Tamai I. Functional pleiotropy of organic anion transporting polypeptide OATP2B1 due to multiple binding sites. *Drug Metab Pharmacokinet* 2012;27(3):360-364.

[36] Kim E, Sy-Cordero A, Graf TN, Brantley SJ, Paine MF, Oberlies NH. Isolation and identification of intestinal CYP3A inhibitors from cranberry (*Vaccinium macrocarpon*) using human intestinal microsomes. *Planta Med* 2011;77(3):265-270.

[37] Roth M, Araya JJ, Timmermann BN, Hagenbuch B. Isolation of modulators of the liver-specific organic anion-transporting polypeptides (OATPs) 1B1 and 1B3 from *Rollinia emarginata* Schlecht (Annonaceae). *J Pharmacol Exp Ther* 2011;339(2):624-632.

[38] de Lima Toccafondo Vieira M, Huang SM. Botanical-drug interactions: a scientific perspective. *Planta Med* 2012;78(13):1400-1415.

[39] Chen C, Cancalon P, Haun C, Gmitter Jr. F. Characterization of furanocoumarin profile and inheritance toward selection of low furanocoumarin seedless grapefruit cultivars. *J Amer Soc Hort Sci* 2011;136(5):358-363.

[40] Greenblatt DJ, Zhao Y, Hanley MJ, et al. Mechanism-based inhibition of human cytochrome P450-3A activity by grapefruit hybrids having low furanocoumarin content. *Xenobiotica* 2012; 42(12):1163-1169.

CHAPTER 3

BIOACTIVITY-GUIDED FRACTIONATION OF GRAPEFRUIT (*Citrus* × *paradisi* Macfad.) JUICE AND EVALUATION OF REPRESENTATIVE COMPONENTS AS INHIBITORS OF AN INTESTINAL ORGANIC ANION-TRANSPORTING POLYPEPTIDE

OVERVIEW

The grapefruit juice (GFJ)-mediated decrease in drug absorption *via* inhibition of intestinal organic anion transporting polypeptides (OATPs) is one of the most widely studied transporter-based dietary substance-drug interactions. Such interactions have been shown to be clinically relevant, manifesting as a decrease in systemic drug exposure. Some compounds in GFJ have been evaluated as OATP inhibitors *in vitro*, but myriad others remain unassessed. The complex composition of GFJ poses many challenges, and bioactivity-guided isolation can be used to identify OATP inhibitors in a systematic manner. Organic-soluble fractions generated from an extract of a commercially available GFJ product, designated as A-M, were evaluated as inhibitors of OATP2B1-mediated uptake using stably transfected Madin-Darby canine kidney type II (MDCKII) cells and estrone 3-sulfate as the probe substrate. Fractions A, B, K, and M were the most potent, inhibiting OATP2B1 activity by ≥ 80% at 10 or 50 μg/mL compared to vehicle control (1.7% methanol). Three subfractions generated from fraction M (m3, m4, m5) inhibited activity by ~60-100% at 10 μg/mL. The most potent pools (3 and 4) from subfractions m4 and m5 inhibited activity by > 85% at 2 μg/mL. A partial structure containing a furanocoumarin moiety was identified in subfraction m3. However, complete structure characterization of this compound and further fractionation of other active pools was not possible due to insufficient starting material. Accordingly, IC_{50}s were determined for known compounds representative of three classes to demonstrate subsequent experiments that would have been initiated had adequate material been available. The compounds tested were the flavanones naringin,

A portion of this chapter will be submitted to *Drug Metabolism and Disposition* as a Short Communication and is presented in the style of the journal.

naringenin, and hesperidin; the furanocoumarins bergamottin and 6',7-dihydroxybergamottin (DHB); and the polymethoxyflavones nobiletin and tangeretin. Nobiletin was the most potent, with an IC_{50} < 5 μM. DHB, naringin, naringenin, and tangeretin were moderately potent, with IC_{50}s ~20-50 μM. Bergamottin and hesperidin were the least potent, with IC_{50}s > 300 μM. IC_{50}s below or within the reported range of concentrations in GFJ support all compounds except bergamottin as candidate inhibitors of intestinal OATP.

INTRODUCTION

The influence of phytochemicals on pharmacologic activity of drugs *via* modulation of absorption and/or elimination processes mediated by metabolizing enzymes and transport proteins is becoming increasingly recognized [1-3]. Grapefruit juice (GFJ) is an extensively studied phytochemical mixture inhibiting both drug metabolism and transport. When consumed in typical volumes, GFJ acts predominately in the intestine, as exemplified by lack of an effect on the pharmacokinetics of intravenously administered drugs or a lack of an effect on elimination half-life of orally administered drugs [4-9]. Despite confinement of the "GFJ effect" to the intestine, GFJ can alter systemic drug exposure significantly, which in turn can lead to serious adverse effects. Examples include rhabdomyolysis with statins, nephrotoxicity with immunosuppressants, and hypotension with calcium channel blockers [10]. Consequently, numerous drug package inserts carry cautionary statements about concomitant intake with GFJ.

The most rigorously studied mechanism underlying the GFJ effect is inhibition of the prominent intestinal drug metabolizing enzyme cytochrome P450 (CYP) 3A. Although the CYP3A inhibitors in GFJ (furanocoumarins) have been established [11-14], the process by which these compounds were identified was neither systematic nor efficient, demonstrated by the > 15-year span between the first report of a GFJ effect [15] and the pivotal clinical study involving a "furanocoumarin-free" GFJ [16]. A more recently discovered mechanism underlying the GFJ effect is inhibition of organic anion-transporting polypeptides (OATPs) located on the apical membrane of enterocytes, resulting in decreased systemic exposure of drug substrates (*e.g.*, fexofenadine, fluoroquinolones, beta-blockers) [17-19]. Since the pioneering report of the GFJ-fexofenadine interaction [20] individual constituents in GFJ have been evaluated as OATP inhibitors. These compounds can be grouped into classes: flavanones, furanocoumarins, or polymethoxyflavones. The flavanone naringin is the only clinically tested compound [21]. Relative to water, GFJ and an aqueous solution of naringin at the same concentration as that in GFJ (~1200 μM) decreased fexofenadine mean systemic exposure by ~40 and ~20%, respectively, in 12 healthy volunteers. The authors

concluded that naringin was a major enteric OATP inhibitor, but the 50% difference in fexofenadine exposure between GFJ and naringin suggested other constituents contributed to the interaction. Such constituents include the flavanone hesperidin, which inhibited fexofenadine uptake in OATP1A2-transfected cervical cancer cells (HeLa) [21], with an IC_{50} (2.7 μM) that was well within concentrations measured in different brands of GFJ (0.2-117 μM) [22-25]. The furanocoumarins bergamottin and 6',7'-dihydroxybergamottin (DHB) and the polymethoxyflavones tangeretin and nobiletin have been shown to inhibit uptake of estrone 3-sulfate in OATP2B1-transfected human embryonic kidney (HEK) 293 cells [26]. IC_{50}s for the furanocoumarins and polymethoxyflavones (~10 μM) were within concentrations measured in GFJ (0.22-53.5 and 0-78 μM, respectively) [14, 27-30]. Although the tested compounds may contribute to the OATP inhibitory effect, as with the identification of CYP3A inhibitors, a limitation to these independent studies is that seemingly arbitrary constituents were tested.

Identification of bioactive compounds from natural products poses a challenge, as such products are complex mixtures of phytochemical compounds [31]. Bioactivity-directed fractionation is routinely used in natural products drug discovery to isolate novel, active compounds of interest [32, 33]. This established systematic procedure was used to identify enteric CYP3A inhibitors in cranberry [34] and hepatic OATP modulators in the stem bark of the aratiku shrub [35]. The purpose of this work was to use a similar approach to screen and identify OATP inhibitors in GFJ, as well as to determine the IC_{50}s of known GFJ constituents representative of three classes using stably transfected Madin-Darby canine kidney type II cells and the probe substrate estrone 3-sulfate.

METHODS

Materials and chemicals. [^{3}H]Estrone 3-sulfate ammonium salt (54.3 Ci/mmol) was purchased from Perkin Elmer (Waltham, MA). Estrone 3-sulfate potassium salt, bromosulfophthalein (BSP), D-glucose, DHB, bergamottin, naringin, hesperidin, tangeretin, and nobiletin were purchased from Sigma-Aldrich (St. Louis, MO). Hanks' balanced salt solution (HBSS) with calcium and magnesium was purchased from Mediatech Inc. (Hendon, VA). Phosphate-buffered saline (PBS), fetal bovine serum, trypsin-EDTA, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L D-glucose, 2 mM L-glutamine, and 110 mg/L sodium pyruvate were purchased from Invitrogen (Carlsbad, CA). Methanol, ethyl acetate, chloroform, *n*-butanol, hexane, scintillation cocktail, sodium hydroxide, and sodium dodecyl sulfate (SDS) 10% solution were purchased from Fisher Scientific (Pittsburgh, PA). Cell culture plates were purchased from Corning Life Sciences (Tewksbury, MA). Madin-Darby canine kidney type II (MDCKII) parental cells and stably transfected MDCKII-OATP2B1 cells were provided by Dr. Markus Grube (Ernst-Moritz-Arndt University, Greifswald, Germany).

Juice extraction and fractionation. Preliminary evaluation of not-from-concentrate (fresh-squeezed, no added water, sugar, or preservatives) GFJ brands included inhibitory potency and ease of access to sufficient commercially available quantities. Five cartons of Florida's Natural® Original Ruby Red 100% Pure Florida Grapefruit Juice (1.75 L each, 8.75 L total) were purchased from a local grocery store. Each carton of juice was divided evenly between six 500-mL polypropylene bottles, with approximately 292 mL of juice in each bottle. After adding 200 mL of ethyl acetate, the bottles were shaken vigorously and centrifuged (5000 × *g* for 10 min at 25 °C). The upper organic layer was transferred to a 5-L round bottom flask. This extraction process was repeated two more times for a total of three extractions for each carton. The ethyl acetate extract was dried *in vacuo* and dissolved in 2 L of a 4:1:5 chloroform:methanol:water (chloroform:methanol:water, v/v/v) mixture and stirred for 1 hour. Both layers were dried *in vacuo*. The dried aqueous layer was resuspended in 1 L of water and shaken with an equal volume of *n*-butanol. Both of these layers were dried *in vacuo*, producing an

organic fraction, an aqueous fraction, and a butanol fraction of the original ethyl acetate extract. The organic fraction was separated into 13 fractions (designated A through M) *via* normal-phase flash column chromatography and tested in the MDCKII-OATP2B1 system. Fractions considered "active" (*i.e.*, ≥ 50% inhibition of estrone 3-sulfate uptake) were selected for further fractionation *via* preparatory high pressure liquid chromatography (HPLC) and tested in the MDCKII-OATP2B1 system. Subsequent active fractions were selected for further fractionation *via* preparatory HPLC. All fractions were resuspended in methanol to yield a stock solution of 2 or 15 mg/mL prior to testing.

Chromatography and isolation. The flash column chromatography separation utilized a Teledyne ISCO CombiFlash Rf System (Teledyne-Isco, Lincoln, NE) equipped with a photodiode array detector (PDA), an evaporative light scattering detector (ELSD), and a 40 g Silica Gold silica gel column (Teledyne-Isco). The mobile phase (hexane, chloroform, and methanol) was run as follows at a linear gradient with a flow a rate of 40 mL/min: 100% hexane to 70% chloroform over 12 min, then increased to 100% chloroform over 10.8 min and maintained for 8.6 min. While hexane remained at 0%, methanol was increased to 1% over 11.6 min then to 5% in 6.2 min, followed by an increase to 10% in 6.3 min, then 20% in 3.8 min. Methanol was increased to 100% over 2.7 min and held for 12 min.

HPLC separations were accomplished *via* a Varian HPLC system equipped with an autosampler, PDA, and ELSD; data were collected and analyzed using Galaxie Workstation software (Varian, Inc., Palo Alto, CA). All mobile phases consisted of methanol (A) and water (B). Separation of fractions A, B, and K utilized a YMC ODS-A C18 preparatory-scale column (250 mm × 20 mm i.d., 5 µm; Waters, Milford, MA). The mobile phase (7 mL/min) began with 40% A, was increased linearly to 100% A over 30 min, then was held for an additional 30 min. Separation of fraction M utilized a Phenomonex Synergi-Max C-12 preparatory-scale column (250 mm × 21.2 mm i.d., 4 µm; Phenomenex, Inc., Torrance, CA). The mobile phase (13 mL/min) began with 50% A, was increased linearly to 100% A over 15 min, then was held for an additional 15 min. Separation of subfractions m4 and m5 utilized a Phenomonex Synergi-Max C-12 semiprep-scale column (250 mm × 10 mm i.d.,

4 μm). The mobile phase (4 mL/min) for separation of m4 began at 70% A, was increased linearly to 100% A over 15 min, then was held for an additional 5 min. The mobile phase (4 mL/min) for separation of m5 began with 80% A, was increased linearly to 100% A over 15 min, then was held for an additional 15 min.

Partial identification of OATP2B1-inhibitory constituent in m3. The partial structure of a major constituent in subfraction m3 was characterized by ultraviolet (UV) absorption, nuclear magnetic resonance (NMR), and high-resolution mass spectrometry (HRMS). UV was determined using a Waters Acquity ultrahigh pressure liquid chromatography (UPLC) system equipped with a PDA and ELSD. NMR spectra were generated using a JEOL ECA-500 NMR (JEOL USA, Inc., Peabody, MA) operating at 500 MHz. High resolution mass spectra were generated using a Thermo LTQ Orbitrap XL mass spectrometer (ThermoFisher, Breman, Germany) equipped with an electrospray ionization source and coupled to a Waters Acquity UPLC system (Milford, MA) equipped with a PDA.

Cell culture conditions. Parental MDCKII and stably transfected MDCKII-OATP2B1 cells were cultured and maintained in DMEM supplemented with 10% fetal bovine serum at 37°C and 5% CO_2 as described previously [36]. Cells were seeded onto 48-well (for screening assays) or 24-well (for IC_{50} determinations) plates at a density of 5×10^4 cells per well and grown to confluence for 2-3 days prior to experimentation.

Screening of GFJ fractions and subfractions for OATP2B1 inhibitory activity. Cells were washed and preincubated for 30 min at 37°C in uptake buffer (HBSS supplemented with 25 mM D-glucose and 10 mM HEPES, pH 7.4). Initial screening was run at pH 7.4 as a baseline condition. Buffer was replaced with a dosing solution (200 μL) consisting of radiolabeled plus unlabeled estrone 3-sulfate (total concentration, 0.5 μM) and GFJ fraction (2, 10, 50, or 250 μg/mL), the OATP inhibitor BSP (250 μM), or vehicle (0.5-2.5% methanol). After 2 minutes at 37°C, cells were washed three times with ice-cold PBS and lysed with 0.1 N sodium hydroxide/0.1% SDS. Liquid scintillation cocktail (5 mL) was added to 200-μL aliquots of the cell lysates, and radioactivity was measured by

scintillation counting. Protein concentrations were determined with a BCA assay kit (Thermo Fisher Scientific, Waltham, MA). Estrone 3-sulfate uptake was linear over the 2-min incubation (data not shown).

IC_{50} determination. Cells were washed and preincubated for 30 min at 37°C in uptake buffer (125 mM NaCl, 48 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 12 mM $MgSO_4$, and 25 mM MES, pH 6). Experiments were run at pH 6 to reflect the characteristic microenvironment at the intestinal brush-border membrane and reports of higher OATP transport activity at acidic extracellular pH [37-39]. Buffer was replaced, and cells were treated at 37°C with a dosing solution (200 μL) consisting of radiolabeled plus unlabeled estrone 3-sulfate (total concentration, 1 μM) and GFJ constituent (0 to 316 μM). After 3 minutes, cells were washed three times with ice cold PBS and lysed with 0.1 N sodium hydroxide/0.1% SDS. The lysates were processed further as described above for the screening assays.

Data analysis. *Calculation of net uptake activity*. Uptake was normalized with respect to protein content. OATP2B1-mediated net uptake was calculated by subtracting uptake in parental cells from that in OATP2B1-expressing cells incubated under parallel conditions. The percent of control OATP activity was calculated by dividing net uptake from experiments in the presence of inhibitor by net uptake from experiments in the presence of vehicle and multiplying by 100. *Apparent IC_{50} determination*. Initial estimates of apparent IC_{50}s were determined from linear regression of net uptake activity vs. natural logarithm of GFJ constituent concentration data. IC_{50}s were determined by fitting either the inhibitory E_{max} model equation 1 or 2 with untransformed data using WinNonlin (v5.2.1, Pharsight, Mountain View, CA) and assessing appropriatness of fit from visual check of observed and predicted data, distribution of residuals, Akaike information criteria, and standard errors.

(1) $$E = E_0 - \frac{I_{max} * C}{IC_{50} + C}$$

(2) $$E = E_0 - \frac{I_{max} * C^{\gamma}}{IC_{50} + C^{\gamma}}$$

where E and E_o are maximum and baseline effects, respectively; I_{max} is the maximum inhibitory effect; C is inhibitor concentration; and γ is the Hill coefficient.

Statistical analysis. Data are presented as means ± standard deviations of triplicate determinations. IC_{50}s are presented as estimates ± standard error of the estimates.

RESULTS

Screening of grapefruit juice fractions as inhibitors of OATP2B1-mediated uptake. Screening consisted of five rounds of testing (Fig. 3.1). The organic-soluble fraction yielded 13 active pools (Fig. 3.2A). Four fractions (A, B, K, M) were the most potent, inhibiting estrone 3-sulfate uptake activity by 77-80% and 81-88% at 10 and 50 μg/mL, respectively, relative to control (Fig. 3.2B). Fraction A produced 9 subfractions, designated a1 to a9; fraction B generated 8 subfractions, designated b1 to b8; fraction K yielded 4 subfractions, designated k1 to k4; and fraction M produced 6 subfractions, designated m1 to m6 (Fig. 3.1). Subfractions considered to be potent were a6, b5, b6, k4, m3, m4, and m5, all of which inhibited estrone 3-sulfate uptake by ≥ 50% at 10 μg/mL (Fig. 3.2C). The remaining subfractions inhibited activity up to 20% or stimulated activity up to 600% of control at 2 μg/mL. Subfractions m3, m4, and m5 inhibited OATP2B1 activity by ~60-100% at 10 μg/mL (Fig. 3.2C). m4 and m5 were available in sufficient quantities for further fractionation via preparatory HPLC (Fig. 3.2C). m4 produced 4 pools; pools 3 and 4 inhibited activity by 69 and 86%, respectively, at 2 μg/mL (Fig. 3.2D). m5 produced 6 pools; pools 3 and 4 inhibited activity by 85 and 80%, respectively, at 2 μg/mL (Fig. 3.2D).

Partial identification of an OATP2B1 inhibitor in a selected grapefruit juice subfraction. Subfraction m3 inhibited estrone 3-sulfate uptake by ~60% at 10 μg/mL (Fig. 3.2C). In contrast to the more potent m4 and m5 subfractions, m3 was relatively pure (70%) and consisted primarily of one compound (Fig. 3.3A). Thus, structure elucidation studies were pursued. UV absorption, NMR, and HRMS studies yielded the partial structure of an inhibitory constituent residing in m3. The UV absorption spectrum of m3 (Fig. 3.3B) exhibited maxima at or near 200, 250 (with a shoulder at ~265), and 310 nm, similar to those in the UV spectrum of DHB, indicating the presence of a furanocoumarin moiety (Fig. 3.3C). The ^{1}H NMR spectrum of m3 showed peaks (red dots) diagnostic of the furanocoumarin structure (Fig. 3.3D). The peaks in the spectrum of m3 (red and green dots) agreed with the peaks in the spectrum of DHB, suggesting that the constituent in m3 is composed of one or more compounds with DHB structures (Fig. 3.3C). Mass spectrometry analysis

revealed that the presumed molecular mass $[M-H]^-$ of m3 is 1113.4, equivalent to 3× the mass of DHB (371.1) minus several protons (Fig. 3.3E). A peak at *m/z* 201 in m3 corresponded to a furanocoumarin moiety and had appeared in all furanocoumarin standards analyzed using the Orbitrap MS (data not shown).

IC_{50} determination for representative compounds in grapefruit juice. IC_{50}s for representative GFJ constituents were determined in OATP2B1-transfected cells (Fig. 3.4). Nobiletin was the most potent, with an IC_{50} of < 5 μM (Fig. 3.5G). Naringenin was moderately potent, with an IC_{50} of ~20 μM (Fig. 3.5D). DHB (Fig. 3.5A), naringin (Fig. 3.5C), and tangeretin (Fig. 3.5F) were approximately equipotent, with IC_{50}s ranging from ~34 to 40 μM. Bergamottin (Fig. 3.5B) and hesperidin (Fig. 3.5E) were the least potent, with IC_{50}s > 300 μM.

DISCUSSION

An increasing number of intestinal (and hepatic) OATP modulators have been identified in botanically-derived foods and supplements [35, 40-43], creating a rich source of potentially novel OATP inhibitors. Although bioactivity-guided isolation is used primarily to identify new therapeutics from natural sources, this approach was used in the current work with the goal of isolating and identifying compounds in GFJ that inhibit an intestinally expressed drug uptake transport protein, specifically OATP2B1. An extract of GFJ was separated into discrete fractions, which were tested in a relevant *in vitro* biosystem. A limitation of this approach is loss of less potent OATP-inhibitory compounds whose effects might be masked in the early stages of fractionation due to the large number of compounds in each fraction. However, early elimination of weak OATP inhibitors increases the efficiency of this method. Fraction M contained potent subfractions (m4 and m5), which inhibited OATP2B1-mediated uptake of estrone 3-sulfate by > 80% at the lowest concentration tested (2 μg/mL), indicating the presence of strong OATP inhibitors in GFJ. Subfraction m3 also was reasonably potent, inhibiting estrone 3-sulfate uptake by ~60% at 10 μg/mL. The mechanism(s) by which OATP2B1 activity was altered is (are) not known but may involve competitive inhibition, multiple binding sites, steric hindrance, or changes in expression due to transcriptional regulation or post-translational modifications. Although identification of OATP inhibitors was the primary objective, some fractions at low concentrations stimulated estrone 3-sulfate uptake, by as much as 600%. OATP stimulation has been observed with other natural product ingredients [35, 42, 44] and has been suggested as a strategy to enhance drug uptake by OATP-expressing cancer cells [35].

Unexpectedly, due to insufficient juice material, further fractionation, complete structure characterization, and kinetic evaluations of the substractions were not feasible. Adequate quantity of starting material is a pragmatic issue in the fractionation of natural products. The starting material for fractionation is usually the whole plant, which is freeze-dried and combined with solvents [33]. Since the amount and nature of bioactive compounds in fruit can change as a result of processing/manufacturing into juice [45, 46], GFJ was selected as the starting material to reflect the

'real world' product that typically is consumed and clinically tested. A GFJ volume exceeding 100 L, preferably from the same lot, might have been sufficient, but obtaining such a volume might not be possible or efficient. Juice is not ideal material for fractionation due to high content of water, sugar, and preservatives, as well as possible contamination with juices of other fruits [34]. As such, dried whole grapefruit (~ 1 kg) should be considered in future studies.

Subfraction m3 inhibited estrone 3-sulfate uptake by ~60% at 10 μg/mL. A series of UV, NMR, and MS studies of this subfraction revealed a structure with a molecular mass of 1113 g/mol containing a furanocoumarin moiety. The structure may contain additional furanocoumarin moieties. However, as aforementioned, complete structure characterization of m3 was not possible due to insufficient material. Nevertheless, a potent compound was isolated after four rounds of testing. Because the furanocoumarins DHB and bergamottin have been shown to inhibit OATP2B1 activity *in vitro* [26], the activity of the furanocoumarin-containing structure in m3 is not unexpected.

Had sufficient GFJ been available for fractionation and isolation, subsequent procedures would have included purification/scaling up or purchase (if commercially available) of select isolated compounds(s). The kinetic properties of each compound would be characterized in a relevant *in vitro* system. Since such experiments were not feasible with the current work, IC_{50}s of known GFJ constituents were determined in OATP2B1-transfected MDCKII cells. IC_{50}s for most of the constituents, except bergamottin, were up to ~1.5-fold higher than results from inhibition studies in which two concentrations of each constituent (1 and 10 μM in 0.5% dimethyl sulfoxide) were tested in HEK293 cells stably expressing OATP2B1 [26]. The higher values may be due to differences in cell systems (MDCKII *vs.* HEK293) and/or solvents (methanol *vs.* dimethyl sulfoxide). The discordant IC_{50} of bergamottin between the current work and previous report (> 300 *vs.* ~10 μM) also could be explained by the different cell systems and/or solvents used. Another explanation is nonspecific binding to the culture plate or protein binding to components in culture medium. The "stickiness" of bergamottin has been demonstrated in human intestinal microsomal studies in which the unbound fraction could not be recovered due to extensive binding to the ultrafiltration device,

even in the absence of microsomal protein [47]. Binding to protein or cellular components in the culture medium is possible, but cells were washed twice and preincubated in serum-free buffer before initiating uptake experiments. The current work reports hesperidin as a weak OATP2B1 inhibitor (IC_{50} > 300 μM). However, hesperidin has been shown to inhibit uptake of estrone 3-sulfate in OATP2B1-expressing *Xenopus laevis* oocytes at a much lower concentration (IC_{50} 1.92 μM) [48]. The striking difference in IC_{50}s of hesperidin again could be due to different cell systems (MDCKII *vs.* oocyte). The interaction liability may be minimal for an OATP2B1 substrate, as hesperidin concentrations in GFJ up to 117 μM have been reported [22, 24, 25]. However, interactions with orange juice are still possible, as hesperidin is present in concentrations up to ~2 mM [49]. As with naringin, clinical studies are needed to determine *in vivo* effects.

Comparison of the IC_{50}s to the reported concentrations in GFJ supported DHB, naringenin, nobiletin, and tangeretin as additional candidate inhibitors (to naringin) of intestinal OATP (Table 3.1) [16, 21, 22, 24, 27-29, 50-54]. However, comparing inhibitor concentrations in GFJ with concentrations at the site of transport is more appropriate, albeit more challenging, to evaluate *in vivo* interaction potential. If such an approach is to be used to streamline subsequent clinical studies, further *in vitro* and/or animal studies are needed to determine the concentration of each inhibitor present at the site of intestinal uptake.

The prospective and retrospective application of bioactivity-guided fractionation described in two recent reports has important implications for the design and interpretation of botanical-drug interaction studies. The first report is the only one published to date that utilized bioactivity-guided fractionation to isolate modulators of drug uptake transporters. Fractions of the extract from *Rollinia emarginata* were screened in a high throughput manner in Chinese hamster ovary cells stably transfected with OATP1B1 or OATP1B3 to identify hepatic OATP modulators [35, 55]. Inhibitory potencies of isolated compounds were determined, which ranged from 4.2-130 μM. Whether or not these observations translate to the clinic remains to be determined. The second report used a bioactivity-guided fractionation approach to select an appropriate cranberry juice for clinical testing

since one brand does not represent the entire market. Five cranberry juice products ranging from concentrate to fresh pressed preparations were evaluated as inhibitors of CYP3A activity (midazolam 1'-hydroxylation) in human intestinal microsomes. The most potent cranberry juice product (brand E) was selected to test in a proof-of-concept clinical study. Indeed, brand E inhibited intestinal CYP3A activity, as demonstrated by a 30% increase in midazolam systemic exposure (AUC) with no change in half-life [56]. Candidate intestinal CYP3A inhibitors were identified subsequently by fractionating whole cranberry fractions and testing against CYP3A activity in human intestinal microsomes and recombinant CYP3A4 [34]. The relatively potent IC_{50}s (< 10 μM) of the resulting isolated triterpenes (maslinic acid, corosolic acid, ursolic acid) suggested that these compounds may have contributed to the cranberry juice-midazolam interaction observed clinically, although the relative concentration in various products prevent generalizations. Collectively, these two studies demonstrated the efficiency and utility of bioassay-guided isolation to identify, either prospectively or retrospectively, selective drug metabolizing enzyme and transport protein inhibitors in established and newly discovered botanicals.

Efficient, systematic isolation of inhibitory bioactive constituents could lead to the establishment of marker compounds that would improve the study of dietary substance-drug interactions, allowing between-study comparisons, standardization, and robust clinical trial design. To the authors' knowledge, the current work is the first report of bioactivity-guided fractionation used to isolate inhibitors of an intestinal uptake transporter from GFJ. The potency of isolated fractions indicated that GFJ contains constituents in addition to naringin that decrease the absorption of orally administered substrates of OATP. Future studies could extend to other OATP substrates, other fruit juices, and other botanical products of interest.

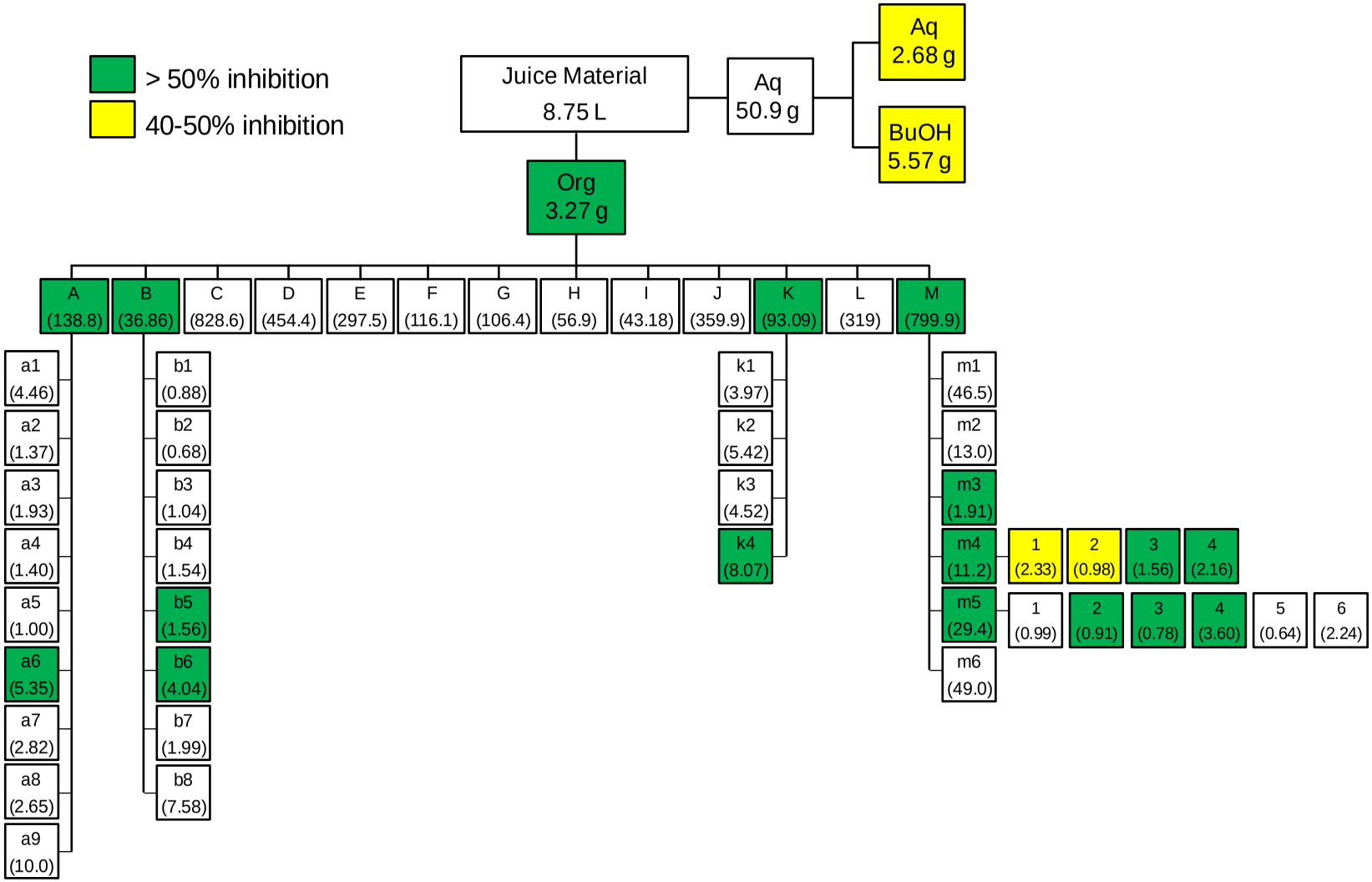


Fig. 3.1. Separation scheme of grapefruit juice extracts. Numbers in parentheses are weights in mg. Aq, aqueous; BuOH, butanol; Org, organic = ethyl acetate.

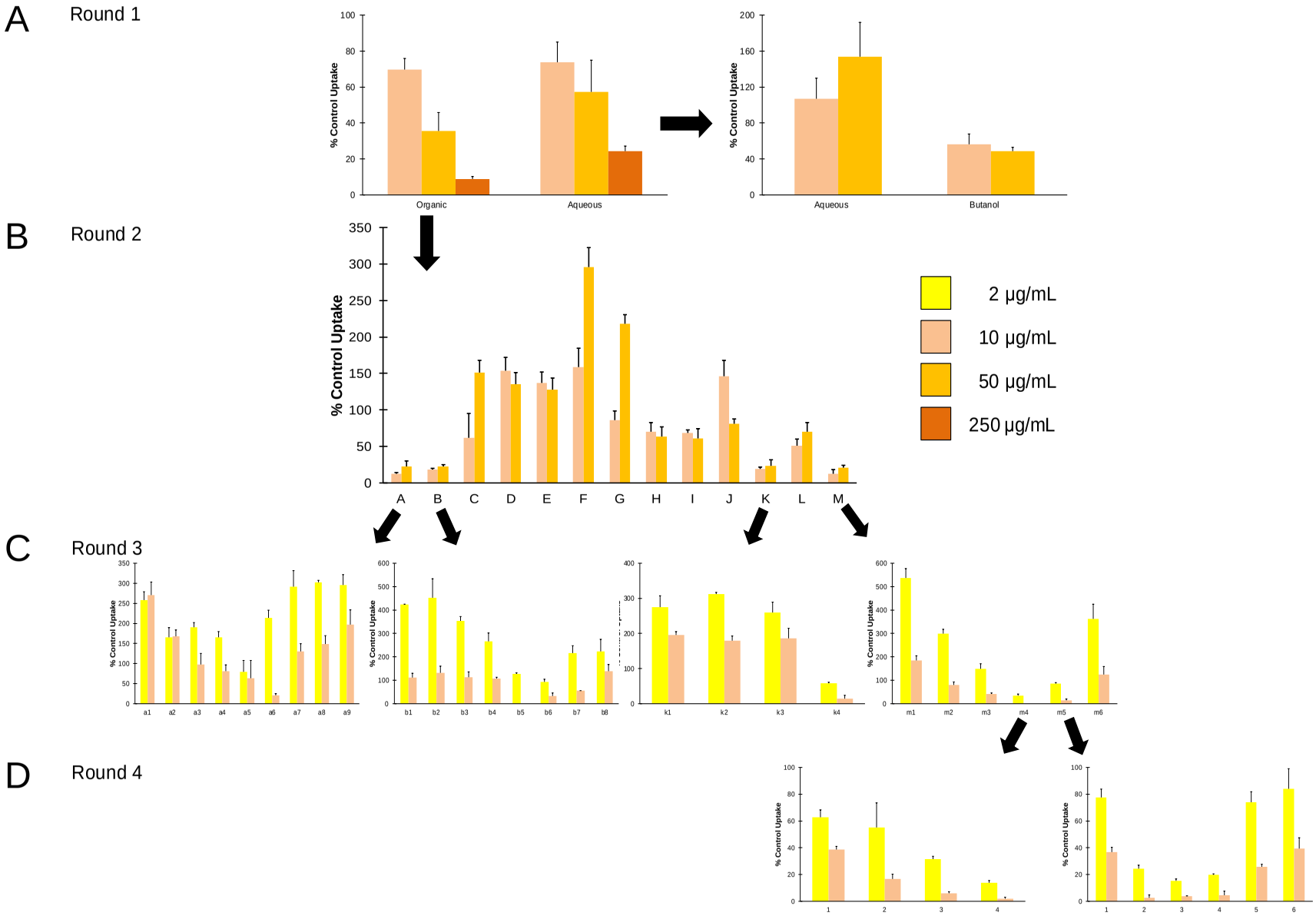
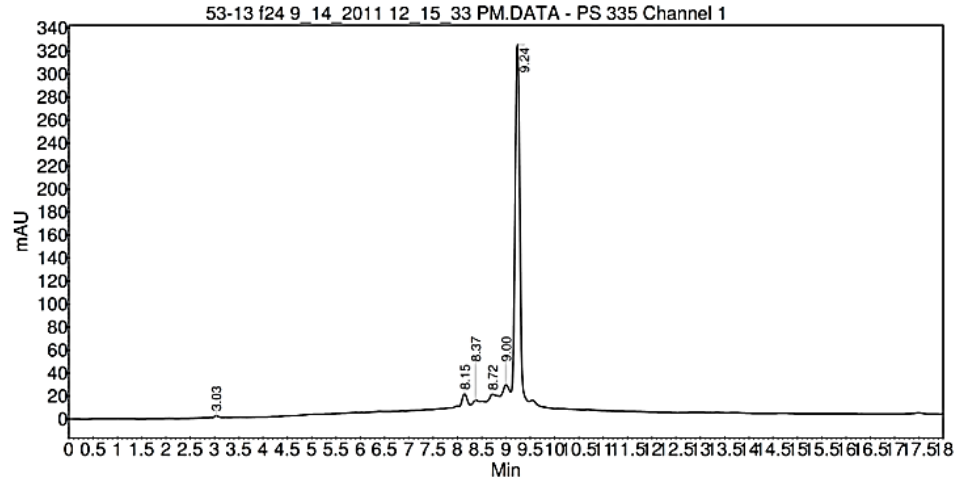
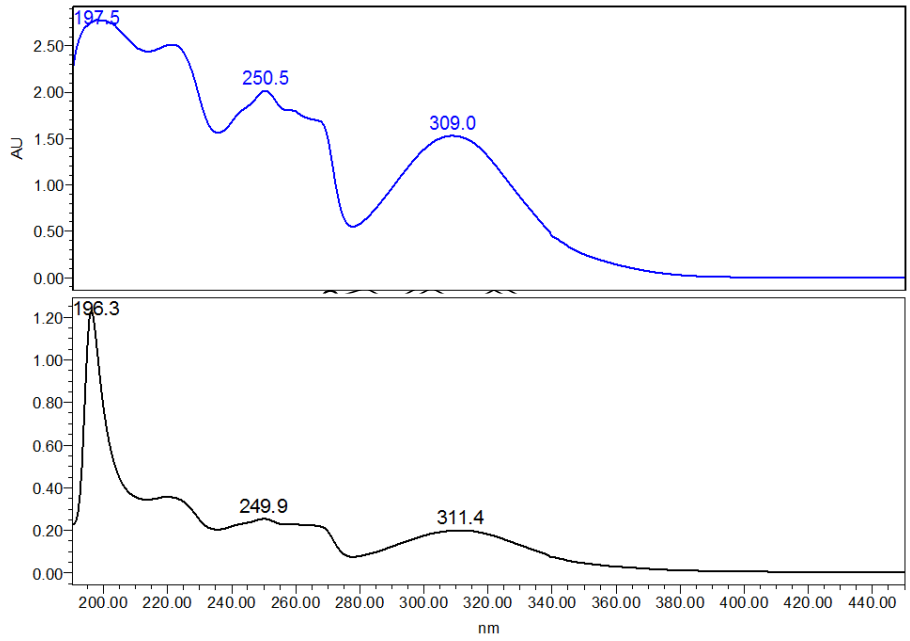

A
Round 1
% Control Uptake
Organic
Aqueous
Aqueous
Butanol
B
Round 2
% Control Uptake
A B C D E F G H I J K L M
2 µg/mL
10 µg/mL
50 µg/mL
250 µg/mL
C
Round 3
% Control Uptake
a1 a2 a3 a4 a5 a6 a7 a8 a9
b1 b2 b3 b4 b5 b6 b7 b8
k1 k2 k3 k4
m1 m2 m3 m4 m5 m6
D
Round 4
% Control Uptake
1 2 3 4
1 2 3 4 5 6

Fig. 3.2. Bioactivity-guided fractionation of grapefruit juice and effects of juice extracts and fractions on OATP2B1-mediated uptake of estrone 3-sulfate *in vitro*. Bars and error bars denote means and SDs from a representative experiment conducted in triplicate. Bromosulfophthalein (250 μM) was used as a positive control for OATP inhibition and inhibited activity by 80-90%.

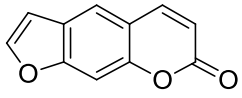
A



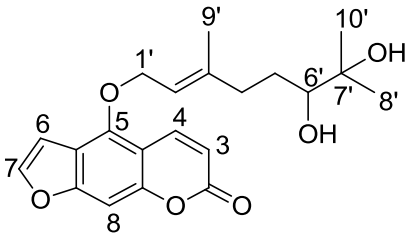
B



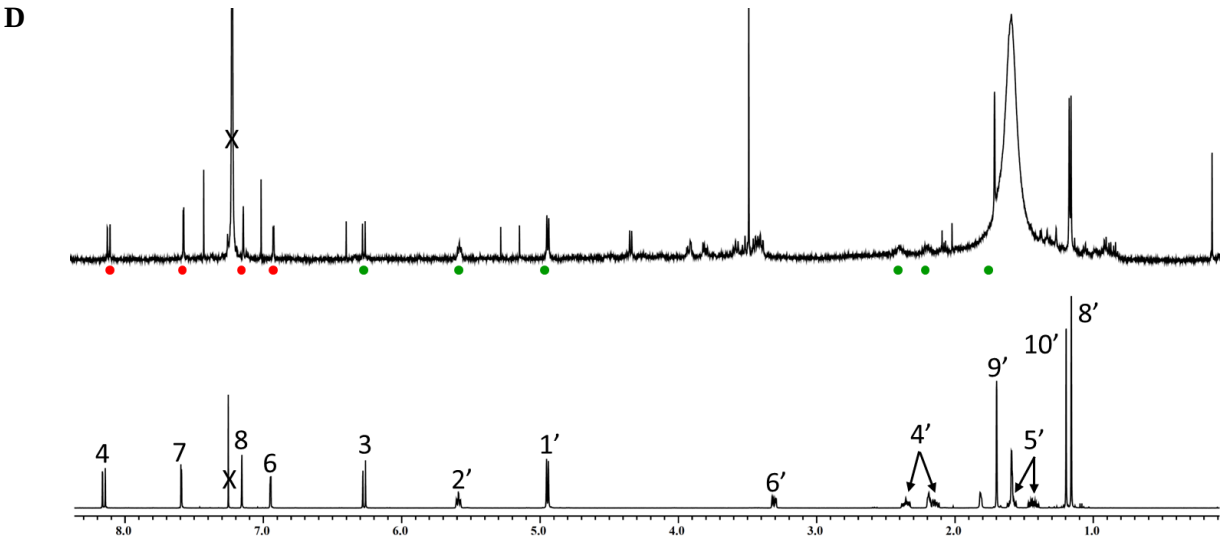
C



Furanocoumarin moiety



DHB
Molecular weight: 372.41 g/mol



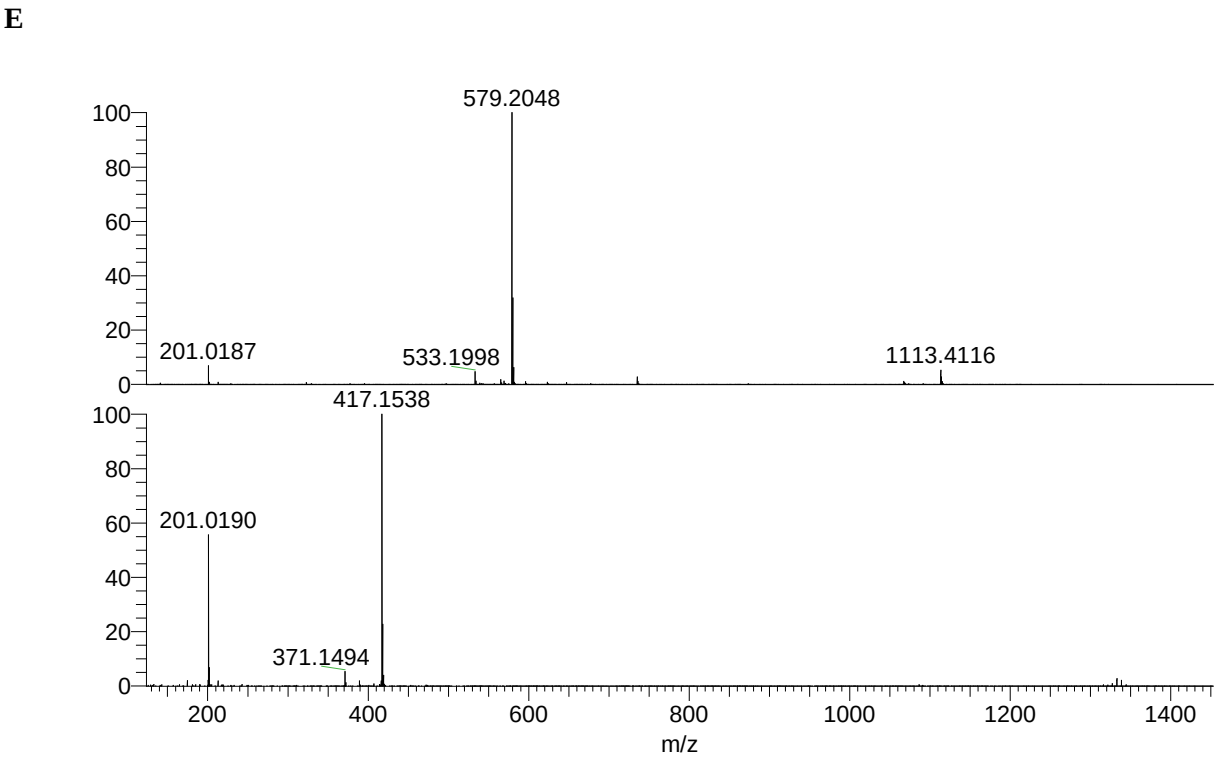
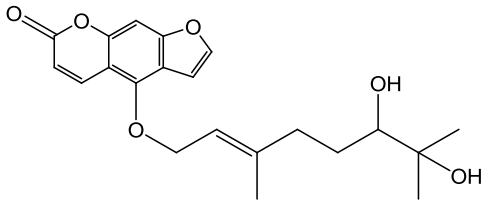
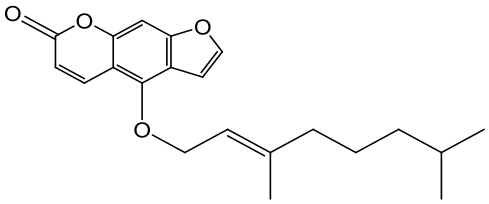


Fig. 3.3. HPLC chromatogram of subfraction m3 (**A**); UV absorption spectra of DHB (upper panel) and m3 (lower panel) (**B**); furanocoumarin moiety and structure of DHB (**C**); NMR spectrum of m3 (upper panel) and DHB (lower panel) (**D**); and HRMS of m3 (upper panel) and DHB (lower panel) (**E**). ●●, peaks diagnostic of furanocoumarin moiety.

Furanocoumarins

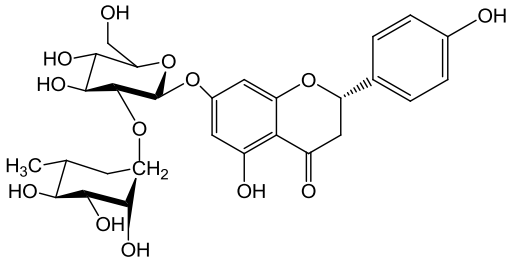


A 6’,7’-Dihydroxybergamottin

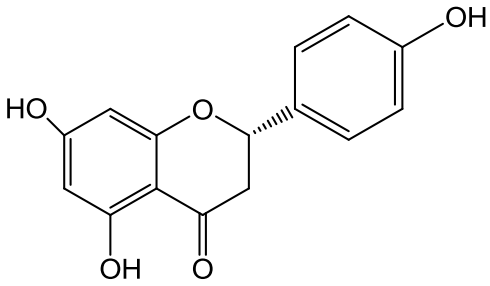


B Bergamottin

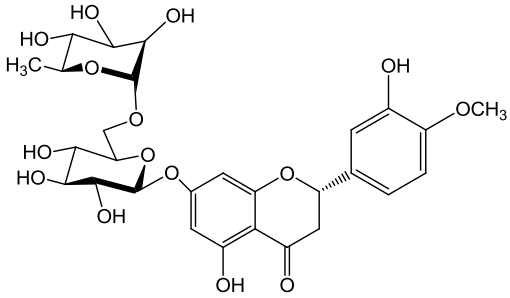
Flavanones



C Naringin

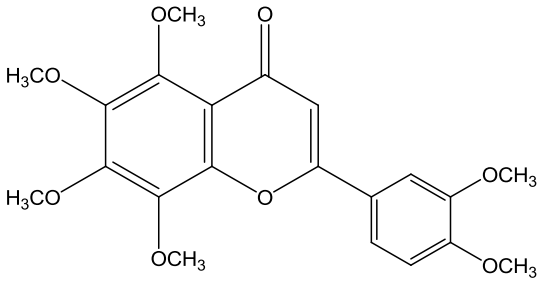


D Naringenin

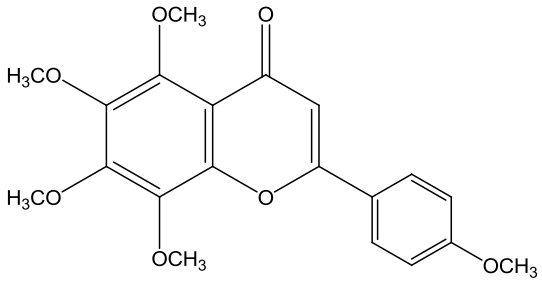


E Hesperidin

Polymethoxyflavones



F Tangeretin



G Nobiletin

Fig. 3.4. Structures of constituents (**A-G**) from representative classes of compounds in grapefruit juice

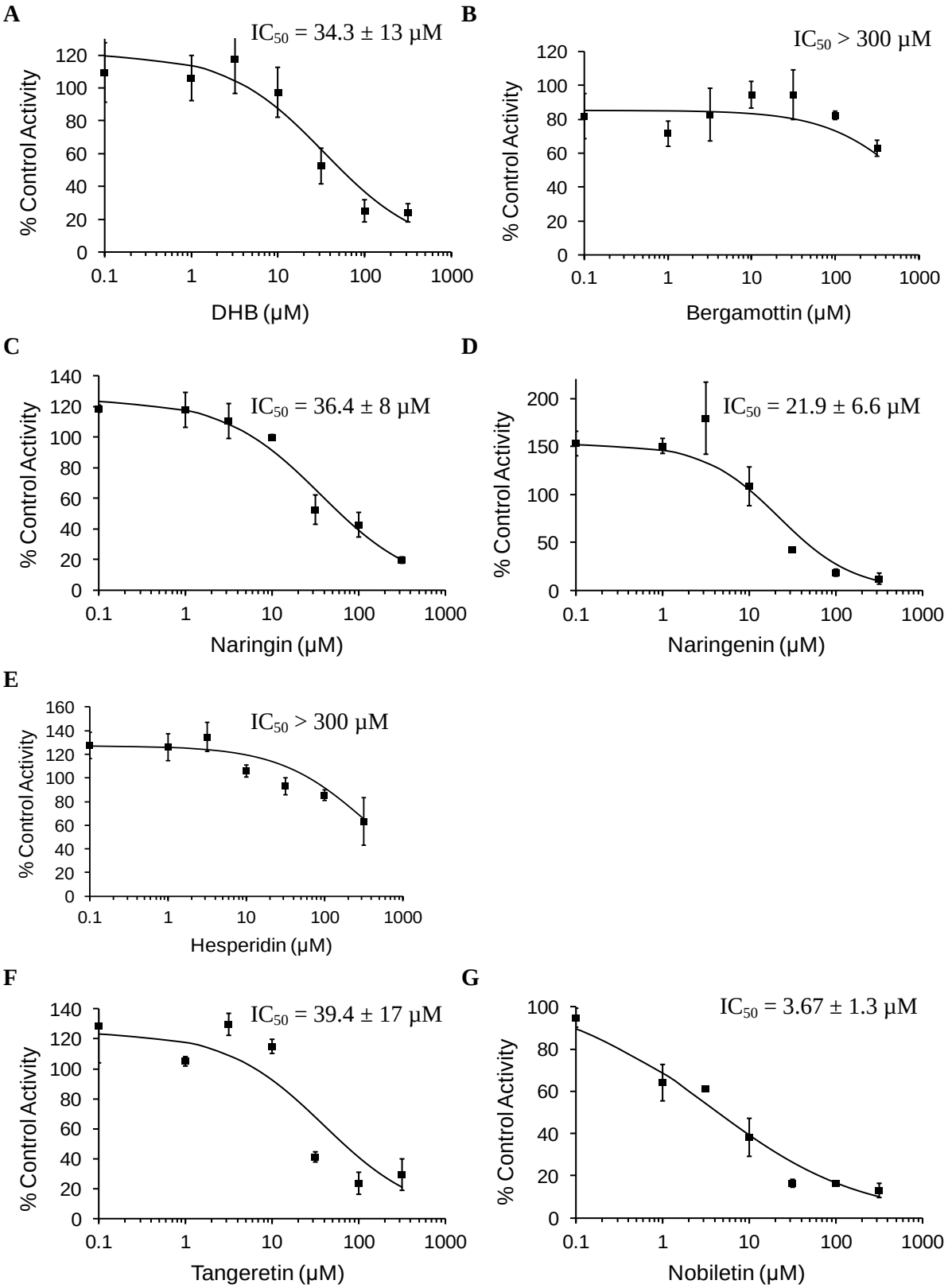

A
IC50 = 34.3 ± 13 µM
% Control Activity
DHB (µM)
B
IC50 > 300 µM
% Control Activity
Bergamottin (µM)
C
IC50 = 36.4 ± 8 µM
% Control Activity
Naringin (µM)
D
IC50 = 21.9 ± 6.6 µM
% Control Activity
Naringenin (µM)
E
IC50 > 300 µM
% Control Activity
Hesperidin (µM)
F
IC50 = 39.4 ± 17 µM
% Control Activity
Tangeretin (µM)
G
IC50 = 3.67 ± 1.3 µM
% Control Activity
Nobiletin (µM)

Fig. 3.5. IC_{50} curves for grapefruit juice constituents representative of three compound classes (**A-G**). Symbols and error bars denote means and standard errors, respectively, of triplicate determinations. Curves denote nonlinear least-squares regression of observed values.

Table 3.1

IC_{50} and concentration of representative flavanones, furanocoumarins, and polymethoxyflavones in grapefruit juice (GFJ)

Constituent	IC_{50} (μM)	I_{GFJ} (μM)[a]	Reference
Flavonoids			
Naringin	36	6540	Brill et al., 2009
Naringenin	22	595	Ho et al., 2000
Hesperidin	>300	117	Uckoo et al., 2012
Furanocoumarins			
6',7'-dihydroxybergamottin	34	52.5	De Castro et al., 2006
Bergamottin	>300	36.3	De Castro et al., 2006
Polymethoxyflavones			
Nobiletin	3.7	28.4[b]	Nogata et al., 2006
Tangeretin	39	78.4[b]	Rouseff and Ting, 1979

[a] I_{GFJ}, highest concentration in GFJ reported

[b] Concentration in flavedo (*i.e.*, peel)

REFERENCES

[1] Rodríguez-Fragoso L, Martínez-Arismendi JL, Orozco-Bustos D, Reyes-Esparza J, Torres E, and Burchiel SW (2011) Potential risks resulting from fruit/vegetable-drug interactions: effects on drug-metabolizing enzymes and drug transporters. *J Food Sci* **76:**R112-R124.

[2] de Lima Toccafondo Vieira M, Huang SM (2012) Botanical-drug interactions: a scientific perspective. *Planta Med* **78:**1400-1415.

[3] Gurley BJ (2012) Pharmacokinetic herb-drug interactions (part 1): origins, mechanisms, and the impact of botanical dietary supplements. *Planta Med* **78:**1478-1489.

[4] Ducharme MP, Warbasse LH, and Edwards DJ (1995) Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. *Clin Pharmacol Ther* **57:**485-491.

[5] Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, and Krähenbühl S (1995) Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther* **58:**20-28.

[6] Lundahl J, Regårdh CG, Edgar B, Johnsson G (1997) Effects of grapefruit juice ingestion-pharmacokinetics and haemodynamics of intravenously and orally administered felodipine in healthy men. *Eur J Clin Pharmacol* **52:**139-45.

[7] Kupferschmidt HH, Fattinger KE, Ha HR, Follath F, and Krähenbühl S (1998) Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man. *Br J Clin Pharmacol* **45:**355-359.

[8] Ebert U, Oertel R, and Kirch W (2000) Influence of grapefruit juice on scopolamine pharmacokinetics and pharmacodynamics in healthy male and female subjects. *Int J Clin Pharmacol Ther* **38:**523-531.

[9] Uno T, Ohkubo T, Sugawara K, Higashiyama A, Motomura S, and Ishizaki T (2000) Effects of grapefruit juice on the stereoselective disposition of nicardipine in humans: evidence for dominant presystemic elimination at the gut site. *Eur J Clin Pharmacol* **56:**643-649.

[10] Bailey DG, Dresser G, and Arnold MO (2012) Grapefruit-medication interactions: forbidden fruit or avoidable consequences? *CMAJ* DOI:10.1503/cmaj.120951.

[11] Edwards DJ, Bellevue FH 3rd, and Woster PM (1996) Identification of 6',7'-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. *Drug Metab Dispos* **24:**1287-1290.

[12] Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME, He K, Lown KS, Woster PM, Rahman A, Thummel KE, Fisher JM, Hollenberg PF, and Watkins PB (1997) Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. *Drug Metab Dispos* **25:**1228-1233.

[13] He K, Iyer KR, Hayes RN, Sinz MW, Woolf TF, and Hollenberg PF (1998) Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. *Chem Res Toxicol* **11:**252-259.

[14] Guo LQ, Fukuda K, Ohta T, and Yamazoe Y (2000) Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity. *Drug Metab Dispos* **28:**766-771.

[15] Bailey DG, Spence JD, Edgar B, Bayliff CD, Arnold JM (1989) Ethanol enhances the hemodynamic effects of felodipine. *Clin Invest Med* **12:**357-362.

[16] Paine MF, Widmer WW, Hart HL, Pusek SN, Beavers KL, Criss AB, Brown SS, Thomas BF, and Watkins PB (2006) A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. *Am J Clin Nutr* **83:**1097-1105.

[17] Greenblatt DJ (2009) Analysis of drug interactions involving fruit beverages and organic anion-transporting polypeptides. *J Clin Pharmacol* **49:**1403-1407.

[18] Bailey DG (2010) Fruit juice inhibition of uptake transport: a new type of food-drug interaction. *Br J Clin Pharmacol* **70:**645-655.

[19] Dolton MJ, Roufogalis BD, and McLachlan AJ (2012) Fruit juices as perpetrators of drug interactions: the role of organic anion-transporting polypeptides. *Clin Pharmacol Ther* doi: 10.1038/clpt.2012.159.

[20] Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, and Kim RB (2002) Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* **71:**11-20.

[21] Bailey DG, Dresser GK, Leake BF, and Kim RB (2007) Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. *Clin Pharmacol Ther* **81:**495-502.

[22] Ross SA, Ziska DS, Zhao K, and ElSohly MA (2000) Variance of common flavonoids by brand of grapefruit juice. *Fitoterapia* **71:**154-161.

[23] Lu Y, Zhang C, Bucheli P, Wei D (2006). Citrus flavonoids in fruit and traditional Chinese medicinal food ingredients in China. *Plant Foods Hum Nutr* **61:**57-65.

[24] Peterson J, Beecher GR, Bhagwat SA, Dwyer J, Gebhardt SE, Haytowitz DB, and Holden JM (2006) Flavanones in grapefruit, lemons, and limes: A compilation and review of the data from the analytical literature. *J Food Comp Anal* **19:**S74-S80.

[25] Uckoo RM, Jayaprakasha GK, Balasubramaniam VM, Patil BS (2012) Grapefruit (*Citrus paradisi* Macfad) phytochemicals composition is modulated by household processing techniques. *J Food Sci* **77:**C921-6.

[26] Satoh H, Yamashita F, Tsujimoto M, Murakami H, Koyabu N, Ohtani H, and Sawada Y (2005) Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos* **33:**518-523.

[27] De Castro WV, Mertens-Talcott S, Rubner A, Butterweck V, and Derendorf H (2006) Variation of flavonoids and furanocoumarins in grapefruit juices: a potential source of variability in grapefruit juice-drug interaction studies. *J Agric Food Chem* **54:**249-255.

[28] Nogata Y, Sakamoto K, Shiratsuchi H, Ishii T, Yano M, Ohta H (2006). Flavonoid composition of fruit tissues of citrus species. *Biosci Biotechnol Biochem* **70:**178-192.

[29] Rouseff RL, Ting SV. Tangeretin content of Florida citrus peel as determined by HPLC (1979) *Proc Fla State Hort Soc* **92:**145-148.

[30] Stremple P (1998) GC/MS analysis of polymethoxyflavones in citrus oils. *J Hi Res Chromatogr* **21:**587-591.

[31] Li Y, Lu J, and Paxton JW (2012) The role of ABC and SLC transporters in the pharmacokinetics of dietary and herbal phytochemicals and their interactions with xenobiotics. *Curr Drug Metab* **13:**624-639.

[32] Oberlies NH and Kroll DJ (2004) Camptothecin and taxol: historic achievements in natural products research. *J Nat Prod* **67:**129-135.

[33] Sarker SD and Latif Z (2006) *Natural Products Isolation*, 2nd ed. Humana Press Inc., New Jersey.

[34] Kim E, Sy-Cordero A, Graf TN, Brantley SJ, Paine MF, and Oberlies NH (2011) Isolation and identification of intestinal CYP3A inhibitors from cranberry (*Vaccinium macrocarpon*) using human intestinal microsomes. *Planta Med* **77:** 265-270.

[35] Roth M, Araya JJ, Timmermann BN, and Hagenbuch B (2011) Isolation of modulators of the liver specific organic anion-transporting polypeptides (OATPs) 1B1 and 1B3 from *Rollinia emarginata* Schlecht (Annonaceae). *J Pharmacol Exp Ther* **339:**624-632.

[36] Grube M, Köck K, Karner S, Reuther S, Ritter CA, Jedlitschky G, and Kroemer HK (2006) Modification of OATP2B1-mediated transport by steroid hormones. *Mol Pharmacol* **70:** 1735-1741.

[37] Kobayashi D, Nozawa T, Imai K, Nezu J, Tsuji A, and Tamai I (2003) Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *J Pharmacol Exp Ther* **306:**703-708.

[38] Nozawa T, Imai K, Nezu J, Tsuji A, and Tamai I (2004) Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J Pharmacol Exp Ther* **308:**438-445.

[39] Leuthold S, Hagenbuch B, Mohebbi N, Wagner CA, Meier PJ, and Stieger B (2009) Mechanisms of pH-gradient driven transport mediated by organic anion polypeptide transporters. *Am J Physiol Cell Physiol* **296:**C570-C582.

[40] Fuchikami H, Satoh H, Tsujimoto M, Ohdo S, Ohtani H, and Sawada Y (2006) Effects of herbal extracts on the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos* **34:**577-582.

[41] Mandery K, Bujok K, Schmidt I, Keiser M, Siegmund W, Balk B, König J, Fromm MF, and Glaeser H (2010) Influence of the flavonoids apigenin, kaempferol, and quercetin on the function of organic anion transporting polypeptides 1A2 and 2B1. *Biochem Pharmacol* **80:**1746-1753.

[42] Roth M, Timmermann BN, and Hagenbuch B (2011) Interactions of green tea catechins with organic anion-transporting polypeptides. *Drug Metab Dispos* **39:**920-926.

[43] Mandery K, Balk B, Bujok K, Schmidt I, Fromm MF, Glaeser H (2012) Inhibition of hepatic uptake transporters by flavonoids. *Eur J Pharm Sci* **46:**79-85.

[44] Wang X, Wolkoff AW, and Morris ME (2005) Flavonoids as a novel class of human organic anion-transporting polypeptide OATP1B1 (OATP-C) modulators. *Drug Metab Dispos* **33:**1666-1672.

[45] Vanamala J, Cobb G, Turner ND, Lupton JR, Yoo KS, Pike LM, and Patil BS (2005) Bioactive compounds of grapefruit *(Citrus paradisi* Cv. Rio Red) respond differently to postharvest irradiation, storage, and freeze drying. *J Agric Food Chem* **53:**3980-3985.

[46] Cancalon PF, Barros SM, Haun C, and Widmer WW (2011) Effect of maturity, processing, and storage on the furanocoumarin composition of grapefruit and grapefruit juice. *J Food Sci* **76:**C543-C548.

[47] Paine MF, Criss AB, and Watkins PB (2004) Two major grapefruit juice components differ in intestinal CYP3A4 inhibition kinetic and binding properties. *Drug Metab Dispos* **32:**1146-1153.

[48] Shirasaka Y, Shichiri M, Mori T, Nakanishi T, and Tamai I (2012) Major active components in grapefruit, orange, and apple juices responsible for OATP2B1-mediated drug interactions. *J Pharm Sci* doi: 10.1002/jps.23357.

[49] Tomás-Barberán FA and Clifford MN (2000) Flavanones, chalcones and dihydrochalcones – nature, occurrence and dietary burden. *J Sci Food Agric* **80:**1073-1080.

[50] Ho PC, Saville DJ, Coville PF, and Wanwimolruk S (2000) Content of CYP3A4 inhibitors, naringin, naringenin and bergapten in grapefruit and grapefruit juice products. *Pharm Acta Helv* **74:**379-385.

[51] Wanwimolruk S and Marquez PV (2006) Variations in content of active ingredients causing drug interactions in grapefruit juice products sold in California. *Drug Metabol Drug Interact* **21:**233-243.

[52] Gattuso G, Barreca D, Gargiulli C, Leuzzi U, and Caristi C (2007) Flavonoid composition of *Citrus* juices. *Molecules* **12:**1641-1673.

[53] Paine MF, Widmer WW, Pusek SN, Beavers KL, Criss AB, Snyder J, and Watkins PB (2008) Further characterization of a furanocoumarin-free grapefruit juice on drug disposition: studies with cyclosporine. *Am J Clin Nutr* **87:**863-871.

[54] Brill, S., Zimmermann, C., Berger, K., Drewe, J., & Gutmann, H. (2009). In vitro interactions with repeated grapefruit juice administration-to peel or not to peel? *Planta Med* **75:**332-335.

[55] Gui C, Obaidat A, Chaguturu R, and Hagenbuch B (2010) Development of a cell based high-throughput assay to screen for inhibitors of organic anion transporting polypeptides 1B1 and 1B3. *Curr Chem Genomics* **4:**1-8.

[56] Ngo N, Yan Z, Graf TN, Carrizosa DR, Kashuba AD, Dees EC, Oberlies NH, and Paine MF (2009) Identification of a cranberry juice product that inhibits enteric CYP3A-mediated first-pass metabolism in humans. *Drug Metab Dispos* **37:**514-522.

CHAPTER 4

EVALUATION OF SELECT GRAPEFRUIT JUICE CONSTITUENTS AS MARKER COMPOUNDS PREDICTIVE OF THE GRAPEFRUIT JUICE EFFECT ON DRUG DISPOSITION

OVERVIEW

Purpose: Evaluate the furanocoumarins 6',7'-dihydroxybergamottin (DHB) and bergamottin as candidate marker compounds predictive of CYP3A4-mediated grapefruit juice (GFJ)-drug interactions.

Methods: *In vitro*-, *in vivo*-, and *in silico*-derived parameters associated with the absorption, distribution, metabolism, and excretion of select CYP3A4 substrates (midazolam, felodipine), DHB, and bergamottin were obtained from the literature. Physiologically-based pharmacokinetic (PBPK) models were developed using commercially available software. Interactions with DHB alone or DHB plus bergamottin were simulated and compared with observed data.

Results: DHB alone predicted the magnitude of effect of GFJ on systemic exposure to midazolam and felodipine ($AUC_{GFJ}/AUC_{control}$) within two-fold of observed effects. The addition of bergamottin improved prediction of midazolam exposure to within 10% of the observed AUC ratio. However, felodipine exposure was overpredicted by > two-fold of the observed AUC ratio in five of six cases.

Conclusions: The CYP3A4 substrate and DHB models performed fairly well, predicting AUCs and AUC ratios within two-fold of observed data. However, improvements in predicting absolute concentrations of substrates and inhibitors depend on further studies, especially those evaluating the pharmacokinetics of DHB and bergamottin and determining whether the contribution of bergamottin to the GFJ effect is substrate-dependent. Nevertheless, based on currently available data, PBPK modeling and simulation support continued evaluation of DHB as a marker compound predictive of CYP3A4-mediated GFJ-drug interactions.

This chapter will be submitted to *Pharmaceutical Research* and is presented in the style of the journal.

INTRODUCTION

The prediction and evaluation of dietary substance-drug interactions are pragmatic concerns that can pose challenges for newly approved, and existing, drugs [1-4]. Grapefruit juice (GFJ) is one of the most extensively studied and clinically relevant dietary substances, acting as a perpetrator of drug interactions. GFJ is mentioned specifically in guidances issued by international regulatory agencies [5, 6], as well as in more than 40 drug package inserts [7]. Since the first report of both a pharmacokinetic (PK) and pharmacodynamic (PD) interaction with the calcium channel blocker felodipine [8], GFJ has been shown to enhance the systemic exposure of myriad drugs, all of which undergo significant first-pass metabolism in the intestine by the prominent drug metabolizing enzyme CYP3A4.

GFJ enhances systemic exposure (AUC, C_{max}) to a broad spectrum of oral medications, including several anti-infectives, cardiovascular agents, immunosuppressants, and statins [9, 10]. When consumed in usual volumes, GFJ inhibits intestinal – but not hepatic – CYP3A4 activity, as evidenced by unaltered clearance and half-life of intravenously administered drugs [10]. The lack of an effect on hepatic CYP3A4 may be due to dilution of the causative ingredients to concentrations below effective inhibitory concentrations, and/or extensive binding of the causative ingredients to plasma proteins, in portal blood [10]. Despite the localized inhibition of CYP3A4 by GFJ, the magnitude of effect can be sufficient to elicit adverse reactions [11]. The primary underlying mechanism is associated with reduced intestinal CYP3A4 protein without a decrease in mRNA, indicating accelerated degradation of the enzyme subsequent to mechanism-based inactivation [12]. Since irreversible loss of CYP3A4 requires *de novo* synthesis to restore enzyme activity, this 'GFJ effect' lasts approximately three days [13, 14].

As with natural products in general, GFJ is a mixture composed of several hundred bioactive ingredients, of which a handful has been studied rigorously. Furanocoumarins in GFJ have been established as potent reversible and mechanism-based inhibitors of CYP3A4 activity *in vitro* [16-20]. A definitive clinical study involving a GFJ devoid of furanocoumarins and the model CYP3A4

substrate felodipine demonstrated furanocoumarins, in aggregate, as major mediators of the felodipine-GFJ interaction [15]. Bergamottin and 6',7'-dihydroxybergamottin (DHB) are two extensively studied, and typically abundant, furanocoumarins present in GFJ. Each has been shown to inhibit CYP3A4 activity and to degrade CYP3A4 protein in the human intestine-derived cell line Caco-2 [20]. Effects on CYP3A4 protein mimicked the relatively rapid (within four hours) ~50% decrease in CYP3A4 protein expression observed in duodenal biopsies obtained from a healthy volunteer after GFJ ingestion [16].

The effect of GFJ *in vivo* varies widely among GFJ brands and is volume-, frequency-, and preparation-dependent [5]. Clinically tested GFJ products administered to human subjects are described as 'single' or 'double' strength; GFJ warnings in drug labels caution against 'excessive consumption (> 1.2 L per day)'. Given the highly variable profile of constituents in different GFJ products [21], such descriptions are ambiguous and misleading when attempting to interpret results from clinical interaction studies and to classify GFJ as a 'strong' versus 'moderate' CYP3A4 inhibitor. DHB and bergamottin have been investigated to the extent that they can be considered 'marker' compounds. If these compounds are validated as 'marker' compounds for CYP3A4 inactivation by GFJ, quantification of one or both compound(s) in GFJ could be sufficient to make between-study comparisons, as well as to predict the likelihood and magnitude of an interaction of whole juice with a given substrate.

Various methods have been developed to predict the magnitude of GFJ-drug interactions. Two studies involved similar approaches in which a predictive model for select CYP3A4 inhibitory GFJ ingredients was developed by establishing a correlation between GFJ ingredient concentration and *in vitro* CYP3A4 inhibitory activity [22, 23]. Both studies determined furanocoumarins (DHB, bergamottin, dimeric spiroesters) to be the most significant variables for CYP3A4 inhibition. However, the correlations could have been driven by a few samples having high furanocoumarin content and high inhibitory activity. A different approach involving modeling and simulation was used in two other studies. A compartmental PK/PD model based on irreversible enzyme inactivation

was developed in the first study to describe the GFJ-felodipine interaction [24]. A physiologically-based pharmacokinetic (PBPK) model was developed in the second study to predict the PK of the CYP3A4 substrates midazolam and simvastatin in the presence of DHB [25]. A limitation shared by both studies is a lack of information on DHB, as well as bergamottin, concentration in the clinically administered GFJs. Taken together, a more robust strategy for evaluating one or more marker furanocoumarins as a predictor of a GFJ interaction is necessitated.

PBPK modeling and simulation for drug development purposes has been increasingly accepted in recent years by the the pharmaceutical industry and regulatory bodies to help streamline and expedite decision-making during both pre-clinical and clinical phases of development [26-28]. Significant advances in the predictability of key PK parameters from *in vitro* and *in vivo* data and in the availability of specialized software have improved the utility and accuracy of this approach [29, 30]. Modeling and simulation are not applied routinely to the study of dietary substance-drug interactions, mostly because dietary substances are not regulated in the same manner as drugs, but also due to the complex bioactive ingredient composition of the dietary substance and associated difficulties in modeling such behavior. However, robust knowledge of major ingredients implicated in the effect of a dietary substance would facilitate investigation of the PBPK modeling approach as a viable tool.

The objective of this work was to evaluate the utility of DHB alone and DHB plusbergamottin as (a) marker compound(s) predictive of CYP3A4 substrate-GFJ interactions. A population-based PBPK software program was used to predict single oral dose PK of select CYP3A4 substrates (midazolam, felodipine) in the presence of intestinal CYP3A4 inhibition (by DHB alone and DHB plus bergamottin) in healthy volunteers. Predictions were compared with observations from clinical GFJ-drug interaction studies in which the concentrations of each furanocoumarin were measured in the test juice.

METHODS

Software (Simcyp® Population-based ADME Simulator)

The population-based PBPK software Simcyp® (version 12, Release 1, Simcyp Ltd., Sheffield, United Kingdom) was used for model development and simulations. This simulator is a platform and database for mechanistic modeling and simulation of the absorption, distribution, metabolism, and excretion (ADME) of drugs and drug candidates [28]. The program combines *in vitro* data with demographic, physiologic, and genetic information on different populations to extrapolate to *in vivo* scenarios.

The software ADMET Predictor™ (version 6.0, Simulations Plus, Inc., Lancaster, CA) is used to estimate biopharmaceutically relevant molecular descriptors related to ADME and toxicity of chemical substances based on molecular structures [31]. The program predictions were utilized as inputs for Simcyp®.

Selection of Clinical GFJ-Drug Interaction Studies

Clinical GFJ-CYP3A4 substrate interaction studies were identified by searching articles published between January 1989 to June 2012 in the PubMed electronic database. Studies to be included met the following criteria:

- Substrate metabolism mainly by CYP3A4
- Availability of *in vitro* data
- Availability of human oral PK information (*i.e.*, trial design, concentration-time profile of substrate administered alone and with GFJ)
- Measurement of DHB and/or bergamottin concentration in serving size of test GFJ
- Healthy volunteers

Plasma concentration-time data from the included studies (Table 4.1) were obtained from the principal investigators or were digitized using GetData Graph Digitizer (version 2.24, http://www.getdata-graph-digitizer.com) and superimposed onto simulations.

Model Development: Substrate and Inhibitor Files

Chemical structures of midazolam, felodipine, DHB, and bergamottin were imported as ".mol" files, created with ChemDraw (version 11.0.1, CambridgeSoft, Cambridge, MA), into ADMET Predictor™ to calculate physicochemical properties describing ionization, partitioning, protein binding, and permeability parameters (Fig. 4.1). The required inputs for midazolam were supplied by Simcyp® software; default values were verified against those reported in the literature and accepted (Table 4.2). Compound files for felodipine, DHB, and bergamottin were created in the Simcyp® library using physicochemical properties, *in vitro* biochemical data, and clinical PK parameters obtained from the literature or ADMET Predictor™ (Tables 4.2 and 4.3). Enzyme kinetic parameters characterizing DHB and bergamottin elimination were estimated using ADMET Predictor™ and clinical studies that reported plasma concentrations of DHB and/or bergamottin [32, 33].

Simulations of Clinical Studies

Simulations/predictions employed trial designs consistent with the selected clinical interaction studies with respect to healthy volunteers, age, weight, number of subjects, and proportion of women (Table 4.1). Dosing regimens of midazolam, felodipine, DHB, and bergamottin were set according to clinical protocol (*i.e.*, single dose, fasted state) (Table 4.1). Simulations proceeded for the reported length of biological sample collection time (*i.e.*, 8, 12, or 24 h). The number of trials for each study simulation was set at 10 to assess variability across subject groups.

Data Analysis

The simulated group mean or median AUCs and AUC ratios (DHB/Control, DHB+BG/Control) of midazolam or felodipine in the absence and presence of DHB or DHB plus bergamottin were reported directly from the software. The mean of AUC ratios from the 10 simulated trials was compared to observed ratios. Comparisons were made visually to determine if observed data lay between the 5th and 95th percentiles of the predicted concentration-time profiles. A prediction error was calculated from the difference between each simulated and observed AUC ratio using the following:

(|Simulated – Observed|/Observed) × 100. Observed AUC values were plotted against simulated values to visualize accuracy of the predictions (*i.e.*, within two-fold boundary). Standard deviations or standard errors were reported for all observed AUCs.

Parameter Sensitivity Analysis

Parameter sensitivity analysis was performed for CYP3A substrate input parameters (*e.g.*, dissolution constant, solubility, absorption rate constant, gastric emptying rate, intestinal transit rate, steady-state volume of distribution). Sensitivity analysis also was performed to assess the influence of DHB and bergamottin input parameters (*e.g.*, blood/plasma ratio, fraction unbound in plasma, fraction unbound in enterocytes, fraction absorbed, absorption rate constant, steady-state volume of distribution, V_{max}, K_m) on CYP3A4 substrate systemic exposure. The effect of each parameter of interest on AUC ratio was evaluated over a wide range of values (0.01 – 100-fold).

RESULTS

One GFJ-midazolam study (out of nine) and six GFJ-felodipine studies (out of 17) met inclusion criteria (Table 4.1). The mean (± SD) concentration of DHB and bergamottin in the serving size of GFJ from all studies was 24.6 ± 16.1 μM and 24.5 ± 5.6 μM, respectively. The mean (± SD) amount of DHB and bergamottin was 2.2 ± 1.6 mg and 2.1 ± 0.6 mg, respectively.

The Simcyp® library model for midazolam (Table 4.2) underpredicted mean plasma concentrations over the time-range (mean AUCs within 23% error) (Fig. 4.2, Table 4.4). Simulated mean midazolam AUCs in the presence of DHB (0.75 mg) alone and with bergamottin (2.94 mg) were within 46% and 16% of observed AUCs, respectively. The simulated mean AUC ratio when bergamottin was added to DHB increased from 1.14 to 1.79 (within 8.5% error of 1.65).

The observed AUC for felodipine in the various clinical studies ranged from 39 to 64 nM·h and from 75 to 130 nM·h in the absence and presence of GFJ, respectively (Table 4.5). The model developed for felodipine using input parameters listed in Table 4.2 generally underpredicted median or mean plasma concentrations over the -time ranges (median and mean AUCs within 54% and 25-60% error, respectively) (Fig. 4.3a-f) (Table 4.5). Simulated mean felodipine AUCs in the presence of DHB (0.75-3.75 mg) alone were between 6% and 73% error. The simulated mean AUC ratios in the presence of DHB alone were within 2-fold error (Figure 4.4a). Simulated mean felodipine AUCs with the addition of bergamottin (0.016-2.94 mg) ranged between 11% and 96% error. The simulated mean AUC ratios in the presence of DHB and bergamottin were two- to four-fold higher than observed AUCs (Figure 4.4b). Although the simulated oral lineshape/profiles were consistent with observed data, the maximum plasma concentrations (C_{max}) were underpredicted, and the time to reach C_{max} (t_{max}) occurred one to two hours earlier.

Due to insufficient/conflicting experimental information and/or lack of confidence in certain parameters, particularly those related to DHB and bergamottin, a parameter sensitivity analysis was performed on felodipine, DHB, and bergmottin input parameters. Felodipine systemic exposure was sensitive to dissolution constant, solubility, permeability/absorption rate constant, gastric emptying

rate, and intestinal transit rate. Solubility and absorption rate constant affected felodipine AUC. Permeability/absorption rate constant showed the largest effect, with approximately two-fold lower AUC when permeability/absorption rate constant was decreased by two orders of magnitude.

Sensitivity analysis showed that AUC ratio was influenced by DHB and bergamottin input parameters – fraction unbound in plasma, fraction unbound in enterocytes, and K_m. AUC increased five-fold when K_m was increased from 0.1 to 25 μM. AUC increased two-fold as the fraction unbound in plasma increased from 0.1 to 1.

DISCUSSION

PBPK modeling and simulation, facilitated by a population-based simulator, was used to examine the well-studied furanocoumarins, DHB and bergamottin, as marker compounds predictive of the GFJ effect on the disposition of CYP3A4 substrates. Interaction studies between select CYP3A4 substrates and one or both furanocoumarins were simulated and compared to observed data meeting inclusion criteria. Since the discovery of the "GFJ effect" in 1989, nearly 70 GFJ-drug interaction studies have been evaluated with drugs from a broad spectrum of therapeutic classes. However, the limiting factor in collecting a large sample size of studies was the lack of reporting on the concentration of DHB and/or bergamottin in the clinical test GFJ. The paucity of studies was unexpected given that furanocoumarins were long suspected as the CYP3A4 inhibitors and have been established as the perpetrators in human subjects for more than five years [15]. The highly variable bioactive ingredient composition between manufacturers (and even lots) is recognized as a confounder to clinical study interpretations [21]. Despite these acknowledgements, investigators generally fail to measure just one ingredient in the clinical test GFJ.

PBPK modeling and simulation showed that midazolam and felodipine systemic exposures under control conditions (water or orange juice) were underpredicted. Although the oral pharmacokinetic profile shapes of felodipine were consistent with those of observed plasma concentration-time profiles, felodipine exposures were underpredicted by two-fold, and t_{max} occurred earlier in most cases. This discrepancy may be due to the extended-release formulation of felodipine. The success of an extended-release dosage form depends upon the interaction of drug parameters, such as drug-release rate and gastrointestinal transit time, coupled with physiological parameters including absorption rates in the various sections of the small and large intestine [51]. The ADAM model in Simcyp is used for solid dosage forms, whereas the first-order absorption and ACAT models assume that the drug is in solution at all times. Parameters related to formulation properties (*e.g.*, solubility, degradation constants) likely need further refinement and/or use of a more specialized program (*i.e.*, GastroPlus) may be required.

DHB alone underpredicted (46% error) the effect of whole GFJ on the systemic exposure of midazolam, whereas the addition of bergamottin significantly improved the prediction (16% error). In contrast, DHB alone predicted (6-73% error) the effect of whole GFJ on the systemic exposure of felodipine in all studies but one [36], whereas the addition of bergamottin overpredicted the AUC ratio by two-fold or more in all cases. These simulations with bergamottin are inconsistent with clinical reports speculating that bergamottin does not contribute significantly to the GFJ effect [32, 50]. An *in vitro* comparison of the time-dependent inhibitory properties of DHB and bergamottin in Caco-2 cells demonstrated a marked difference in both the rate of cell entry and time to onset of CYP3A4 inhibition [20]. The more lipophilic bergamottin had a slower rate of entry and a delayed onset of inhibition compared to DHB, suggesting CYP3A4 is maximally inhibited by DHB before bergamottin has an opportunity to act. Clearly, even though DHB and bergamottin belong to the same furanocoumarin class, the compounds behave differently. The inhibition model in Simcyp® allows up to three inhibitors, utilizing an additive equation for multiple inhibitors (with the same mechanism). Although bergamottin exhibits more potent inhibition than DHB *in vitro* [49], the net effect of both *in vivo* is unknown. It is also possible that other furancoumarin derivatives (*e.g.*, dimers) may be more appropriate marker compounds [52]. However, the insufficient *in vitro* data and unknown *in vivo* inhibitory potential of these compounds preclude the proposal of new marker candidates at this time.

The prediction errors could be attributed to several other factors. The accuracy of input parameters that are not derived experimentally is a common limitation in modeling and simulation. Physicochemical properties related to solubility, permeability, and lipohilicity of natural products are not determined routinely, although attempts to classify these products based on intestinal permeability and solubility (akin to the Biopharmaceutical Classification System for pharmaceutical drugs) have been undertaken [31]. Clinically relevant metabolism and transport interaction studies are appearing more frequently in the literature. However, *in vitro* studies still lack evaluations critical to the precision and accuracy of natural product-drug interaction models, such as the fraction unbound in plasma and enzyme kinetic parameters (specifically those describing the metabolism of the natural

product ingredients *per se*). The fraction unbound in plasma, K_m, and V_{max} for DHB and bergamottin were estimated based on structure and physicochemical properties, with some verification using sparse clinical data in which plasma concentrations of DHB were reported [32, 33]. The K_m and V_{max} for DHB were deemed adequate, as simulations of a GFJ-felodipine study in which a "serum" made from GFJ containing DHB (3.41 mg) and a low amount of bergamottin (0.016 mg) were consistent with observed data [38]. In contrast, simulations with the addition of bergamottin slightly overpredicted systemic exposures of midazolam (8%) but substantially overpredicted those of felodipine (up to 270%). This discordance may be due to different CYP3A4 substrate-DHB/bergamottin interactions. Substrates and modifiers of CYP3A4 can have differential effects, which have been attributed to the existence of multiple binding domains within the CYP3A4 active site [53]. Such complex interactions can confound the prediction of *in vivo* interactions from *in vitro* data. A series of *in vitro* studies demonstrated that representative prototypes of the CYP3A4 substrate subgroups (midazolam, testosterone, and nifedipine) show distinct kinetic properties [53]. Felodipine, a structural analog of nifedipine, showed different effects than nifedipine, indicating the possibility of different binding domains on CYP3A4 for these substrates despite similar chemical structures. Based on this information, it is possible that DHB and bergamottin are predictive for midazolam subgroup-GFJ interactions, whereas DHB alone is sufficient to predict felodipine subgroup-GFJ interactions. This hypothesis requires verification by *in vitro* inhibition and binding studies utilizing human intestinal microsomes, felodipine, DHB, and bergamottin. A more likely reason for the overprediction of felodipine by bergamottin is the (inaccurate) estimation of the fraction unbound in plasma and gut, K_m, and V_{max} for bergamottin based on structure and physicochemical properties. Simulations of clinical studies in which felodipine and bergamottin capsules or bergamottin-rich lime juice were administered to healthy volunteers also were overpredicted by > two-fold [32, 50] (data not shown). Although sparse plasma concentration-time data for bergamottin was available [32, 33], attempts to retrieve reliable estimated values for bergamottin were unsuccessful. Additional *in vitro* experiments and full pharmacokinetic profile characterization of the inhibitors (*i.e.*, DHB and bergamottin), along

with the substrates, are needed to discern the impact of one or both. The increasing sensitivity of bioanalytical methods should permit such a characterization in the near future.

Another possibility for the prediction errors is that the model did not account for bergamottin conversion/metabolism to DHB. It has been shown that incorporation of active metabolite (of the inhibitor) data improves drug-drug interaction predictions [54]. A recent report showed that inactivation of CYP3A4 by bergamottin results in formation of a modified apoprotein-CYP3A4 and a reactive metabolite (DHB plus one oxygen atom) [55]. This reactive DHB covalently binds to a glutamine residue (Gln273) and contributes to the mechanism-based inactivation of CYP3A4 by bergamottin. DHB also has been speculated to be further metabolized to another furanocoumarin, bergaptol, and excreted in urine [56]. The metabolic pathways of bergamottin and DHB are not well characterized *in vitro* and *in vivo*, highlighting a critical deficiency of natural product-drug (and drug-drug) interaction prediction assessment. The pharmacokinetic profile of the perpetrator is not routinely characterized in clinical studies, despite that the information is vital to the successful prediction of an interaction. Although the AUC ratio predictions were within two-fold of observed values, the model requires further refinement, mostly related to DHB and bergamottin. Remarkably, even though ketoconazole has been commercially available for nearly 30 years and is used as a prototypic CYP3A inhibitor, little information existed about routes of elimination until recently [57].

An additional explanation for the prediction errors could be the difference in observed clinical study and Simcyp® virtual healthy volunteer populations. The study design conditions and 10 trials per study were duplicated and simulated to address such issues. However, CYP3A5 expressers in the virtual population were excluded from simulations. Although not genotyped, some subjects in the selected GFJ studies could have been CYP3A5 expressers, who presumably would be less sensitive to inhibition [58]. Simcyp contains a number of population libraries with corresponding genotype frequencies for various CYPs, UGTs, and transporters. Subsequent simulations can incorporate CYP3A5 expressers in the virtual population.

To the author's knowledge, this work is one of two PBPK modeling approaches used to predict the effect of GFJ on the pharmacokinetics of CYP3A4 substrates [25]. Although a previous study aimed to predict (retrospectively) midazolam and simvastatin pharmacokinetics using DHB as an inhibitor, a major drawback to the study was the lack of information on DHB concentration in the clinically administered GFJs. An atypical DHB "concentration" of 43 μmoles (~16 mg DHB) was assumed, and the two modeled clinical interaction studies did not report concentrations of DHB and/or bergamottin. Since furanocoumarin composition is thought to be an index to predict GFJ-drug interactions mediated by CYP3A4 inhibition, the *in silico* approach in this work takes this important factor into account. The strategy provides a framework for identifying and evaluating causative ingredients that would lead ultimately to predicting and informing clinical trial design of other CYP3A4 substrate-GFJ interactions.

Concomitant intake of drugs and foods/supplements perceived as healthy and complementary to health is increasing steadily. In addition, use of naturally-derived "pharmacokinetic boosters" (*i.e.*, GFJ) has been proposed to decrease the cost of drugs through improved bioavailability and dose reduction in some patients. For example, GFJ interactions with immunosuppressants and anti-cancer agents have been studied [59-62]. Since GFJ presumably lacks systemic adverse effects that could be encountered with other CYP3A4 inhibitors (*e.g.*, erythromycin, cimetidine, ketoconazole), GFJ has been proposed as an ideal substance. Due to highly variable concentrations of furanocoumarins in GFJ, concomitant intake with a random GFJ product off the grocery store shelf is not recommended. DHB has been marketed as a dietary supplement capable of "enhancing absorption naturally" of other concomitantly ingested supplements. A product with a known, consistent quantity of DHB and/or other potent GFJ inhibitors may be more acceptable. A modeling approach could be useful in predicting pharmacokinetics and assessing the clinical utility of these products in combination with drug therapy.

Despite a massive accumulation of research on GFJ, many questions remain. PBPK modeling and simulation can inform the design of future *in vitro* and *in vivo* studies to predict interactions

prospectively or elucidate possible underlying mechanisms. Although several limitations and knowledge gaps are acknowledged, the approach described in this work underscores the need for appropriate strategies to investigate not just GFJ but other less understood natural products increasingly used as therapeutic agents in combination with drugs.

Table 4.1 Summary of Eligible Clinical Interaction Studies

CYP3A4 Substrate	No. of Subjects	Proportion of Women	Age Range (years)	Weight Range (kg)	Ethnicity	Substrate Dose (mg)	GFJ Volume (mL)	DHB in GFJ (μM)	DHB "Dose" (mg)	BG in GFJ (μM)	BG "Dose" (mg)	Reference
Midazolam	25	0.16	20-46	NR	NR	6	300[a]	6.7	0.75	29	2.94	14
Felodipine	18	0.50	M: 24-63 W: 22-53	M: 60-101 W: 57-129	AA: 1 M, 2W	10	240	30.8	2.75	28.0	2.27	15
	12	0	18-40	NR	Caucasian	10	250	26	2.45[b]	NM	--	33
	12	0.17	M: 18-20 W: 20-40	NR	NR	10	250	42	3.91	28	2.36	34
	12	0	20-27	51-78	Han Chinese	10	250	1.0	0.093	18.8	1.59	35
	10	0.50	21-32	NR	NR	10	240	23	2.05	16	1.29	36
	5	0.60	20-37	65-77	NR	10	240	43	3.75	27	2.18	38

GFJ, grapefruit juice; DHB, 6',7'-dihydroxybergamottin; BG, bergamottin; NM, not measured; NR, not reported (assumed to be 70 kg or Caucasian); M, men; W, women; AA, African American

[a] GFJ administered 2 h before substrate. GFJ ingested with substrate in all other studies

[b] Combined DHB concentration in supernatant (1.85 mg) and particulate (0.6 mg)

Table 4.2 Input Data for Substrate Files in Simcyp®

Property	Midazolam[a]	Felodipine (Method or Reference)
Physicochemical		
Molecular weight (g/mol)	325.8	384.26 (ADMET Predictor)
Log*P*	3.53	4.81 (ADMET Predictor)
Compound type	Ampholyte	Neutral
pK_a 1	10.95	
pK_a 2	6.2	
B/P	0.603	0.70 (39, 40)
$f_{u,plasma}$	0.032	0.0048 (41, 42)
Absorption		
Model type	Advanced Compartmental Absorption & Transit (ACAT)	Advanced Dissolution, Absorption & Metabolism (ADAM)
$f_{u,gut}$	1	1[c]
f_a	1	1 (43)
k_a (h^{-1})	2.5	3.3 (13)
Permeability ($\times 10^{-6}$ cm/s)		
Caco-2 cells	213	22.9 (44)
Distribution		
Model type	Full PBPK	Full PBPK
V_{ss} (L/kg)	4.6[b]	17[b]
Elimination		
Pathway 1	1'-Hydroxylation	Dehydrogenation
$V_{max, CYP3A4}$ (pmol/min/pmol rCYP)	5.23	36.8 (47)
$K_{m, CYP3A4}$ (μM)	2.16	0.94 (47)
$f_{u,inc, CYP3A4}$	1	1
Pathway 2	4-Hydroxylation	
$V_{max, CYP3A4}$ (pmol/min/pmol rCYP)	5.2	
$K_{m, CYP3A4}$ (μM)	31.8	
$f_{u,inc, CYP3A4}$	1	
Additional clearance	Intestine	Intestine
$Cl_{,int}$ (μL/min/pmol rCYP3A)	6.8[d] (40)	23.4 (40)[d]

[a] Simcyp® provided (Sim-Midazolam file) except $Cl_{,int}$

[b] Predicted using Method 2: Rodgers and Rowland [46, 47]

[c] Assumed [43]

[d] Human intestinal microsomes prepared by enterocyte elution

Log*P*, logarithm of the octanol-water partition; pK_a, acid dissociation constant; B/P, blood to plasma partition ratio; $f_{u,plasma}$, unbound fraction of substrate in plasma; $f_{u,gut}$, unbound fraction of substrate in enterocytes; f_a, fraction available to be absorbed from dosage form; k_a, first-order absorption rate

constant; V_{ss}, volume of distribution at steady state using tissue volumes for a population representative of healthy volunteers; V_{max}, maximum rate of metabolite formation; rCYP, recombinant CYP; K_m, Michaelis-Menten constant; $f_{u,inc}$, unbound fraction in incubation; Cl_{int}, intrinsic clearance (uncorrected for $f_{u, inc}$)

Table 4.3 Input Data for Inhibitor Files in Simcyp®

Property	DHB (Method or Reference)	Bergamottin (Method or Reference)
Physicochemical		
Molecular weight (g/mol)	372.42 (ADMET Predictor)	338.41 (ADMET Predictor)
Log*P*	2.79 (ADMET Predictor)	5.44 (ADMET Predictor)
Compound type	Diprotic Acid	Neutral
pK_a 1	13.58	
pK_a 2	12.80 (ADMET Predictor)	
B/P	0.61 (ADMET Predictor)	0.59 (ADMET Predictor)
$f_{u,plasma}$	0.062 (ADMET Predictor)	0.01[a]
Absorption		
Model type	Advanced Compartmental Absorption & Transit (ACAT)	First-Order Absorption
$f_{u,gut}$	1[b]	0.1[c]
f_a	1[a]	0.82[a]
k_a (h^{-1})	2.55[a]	0.57[a]
Permeability ($\times 10^{-6}$ cm/s)		
Caco-2 cells	64 (20)	7.4 (20)
Distribution		
Model type	Full PBPK	Minimal PBPK
V_{ss} (L/kg)	1.69[d]	184.2[d]
Elimination		
$V_{max, rCYP}$ (pmol/min/rCYP)	9 (Estimated)	11 (Estimated)
$K_{m, rCYP}$ (μM)	2 (Estimated)	6 (Estimated)
$f_{u,inc}$	1	0.01[c]
Interaction type		
Competitive Inhibition	CYP3A4	CYP3A4
$K_{i, CYP3A4}$ (μM)	0.5[e] (49)	6.1[e] (49)
$f_{u,inc, CYP3A4}$	0.48 (49)	0.01[c] (49)
Mechanism-Based Inhibition	CYP3A4	CYP3A4
$K_{app, CYP3A4}$ (μM)	1.1 (49)	0.7 (49)
$k_{inact, CYP3A4}$ (h^{-1})	24.6 (49)	42 (49)
$f_{u,inc, CYP3A4}$	0.48 (49)	0.01[c] (49)

[a] Simcyp predicted

[b] Assumed [43]

[c] Assumed

[d] Predicted using Method 2: Rodgers and Rowland [46, 47]

[e] Inhibition kinetics of DHB and bergamottin toward midazolam 1'-hydroxylation activity in recombinant CYP3A4

DHB, 6',7'-dihydroxybergamottin; Log*P*, logarithm of the octanol-water partition; pK_a, acid dissociation constant; B/P, blood to plasma partition ratio; $f_{u,plasma}$, unbound fraction of substrate in plasma; $f_{u,gut}$, unbound fraction of substrate in enterocytes; f_a, fraction available from dosage form; k_a, first-order absorption rate constant; V_{ss}, volume of distribution at steady state using tissue volumes for a population representative of healthy volunteers; V_{max}, maximum rate of metabolite formation; rCYP, recombinant CYP; K_m, Michaelis-Menten constant; $f_{u,inc}$, unbound fraction in incubation; K_i, concentration of inhibitor required to achieve half maximal inhibition; K_{app} or K_I, concentration of mechanism-based inhibitor associated with half maximal inactivation rate; k_{inact}, inactivation rate of enzyme

Table 4.4 Comparison of Observed and Predicted Systemic Midazolam Exposure

	$AUC_{0\text{-}8h}$ (nM·h)				
	Predicted			Observed	
	Mean (SD)	Mean Ratio (SD)		Mean (SE)	Mean Ratio (SE)
		DHB/Control	DHB+BG/Control	GFJ/Control	
Control[a]	154 (63.2)			199 (22.1)	
DHB 0.75 mg	176 (73.7)	1.14 (0.02)		328 (37.1)	1.65 (0.04)
+ BG 2.94 mg	274 (119)		1.79 (0.34)		

SE, standard error; SD, standard deviation; DHB, 6',7'-dihydroxybergamottin; BG, bergamottin; +, DHB plus BG; $AUC_{0\text{-}8}$, area the curve from time zero to 8 h

[a] Greenblatt *et al*., 2003 [14]

Table 4.5 Comparison of Predicted and Observed Systemic Felodipine Exposure

	AUC (nM·h)				
	Predicted			Observed	
	Mean (SD)	Mean Ratio (SD)		Mean (SE)	Mean Ratio (SD)
Reference Treatments		DHB/Control	DHB+BG/Control	GFJ/Control	
Paine et al., 2006 (15)					
Control[a]	25[b, c] (5-100)[d]			54[b, c] (29-150)[d]	
DHB 2.75 mg	67[b, c] (8-238)[d]	2.18[c] (1.43-3.46)[d]		110[b, c] (58-270)[d]	2.03
+ BG 2.27 mg	186[b, c] (36-765)[d]		7.45[c] (2-24)[d]		
Bailey et al., 1998 (34)					
Control	22 (13)			53[e] (7)	
DHB 2.45 mg	43 (30)	1.93 (0.22)	---	130[e] (15)	2.45
Bailey et al., 2000 (35)					
Control	18 (11)			25[f] (5)	
DHB 3.91 mg	51 (32)	2.76 (0.43)		54[f] (8)	2.16
+ BG 2.36 mg	106 (58)		6.39 (2.1)		
Guo et al., 2007 (36)					
Control	33 (17)			64[e] (27)[f]	
DHB 0.093 mg	34 (18)	1.03 (0.01)		126[e] (48)[f]	1.97
+ BG 1.59 mg	112 (56)		3.42 (0.7)		
Malhotra et al., 2001 (37)					
Control[a]	29 (18)			38.6[b] (5.5)	
DHB 2.05 mg	54 (39)	1.80 (0.19)		74.7[b] (8.8)	1.93
+ BG 1.29 mg	123 (82)		4.27 (1.0)		
Kakar et al., 2004 (38)					
Control[a]	25 (14)			39.1[h] (20.4)[g]	
DHB 3.75 mg	70 (39)	2.80 (0.47)		99.5[h] (49.4)[g]	
+ BG 2.19 mg	154 (80)		6.65 (2.2)		4.0 (4.1)[j]

AUC, area the curve; SD, standard deviation; SE, standard error; DHB, 6',7'-dihydroxybergamottin; BG, bergamottin; GFJ, grapefruit juice; +, DHB plus BG; NR, not reported

[a] Control = Orange juice (OJ)

[b] AUC_{0-24}, area the curve from time 0 to 24 h

[c] Median

[d] Range

[e] AUC_{0-12}, area the curve from time 0 to 12 h

[f] AUC_{0-8}, area the curve from time 0 to 8 h

[g] SD

[h] AUC_{0-last}, area the curve from time 0 to time corresponding to last measurable concentration

[i] Aqueous extract of GFJ (serum)

[j] AUC ratio GFJ/OJ

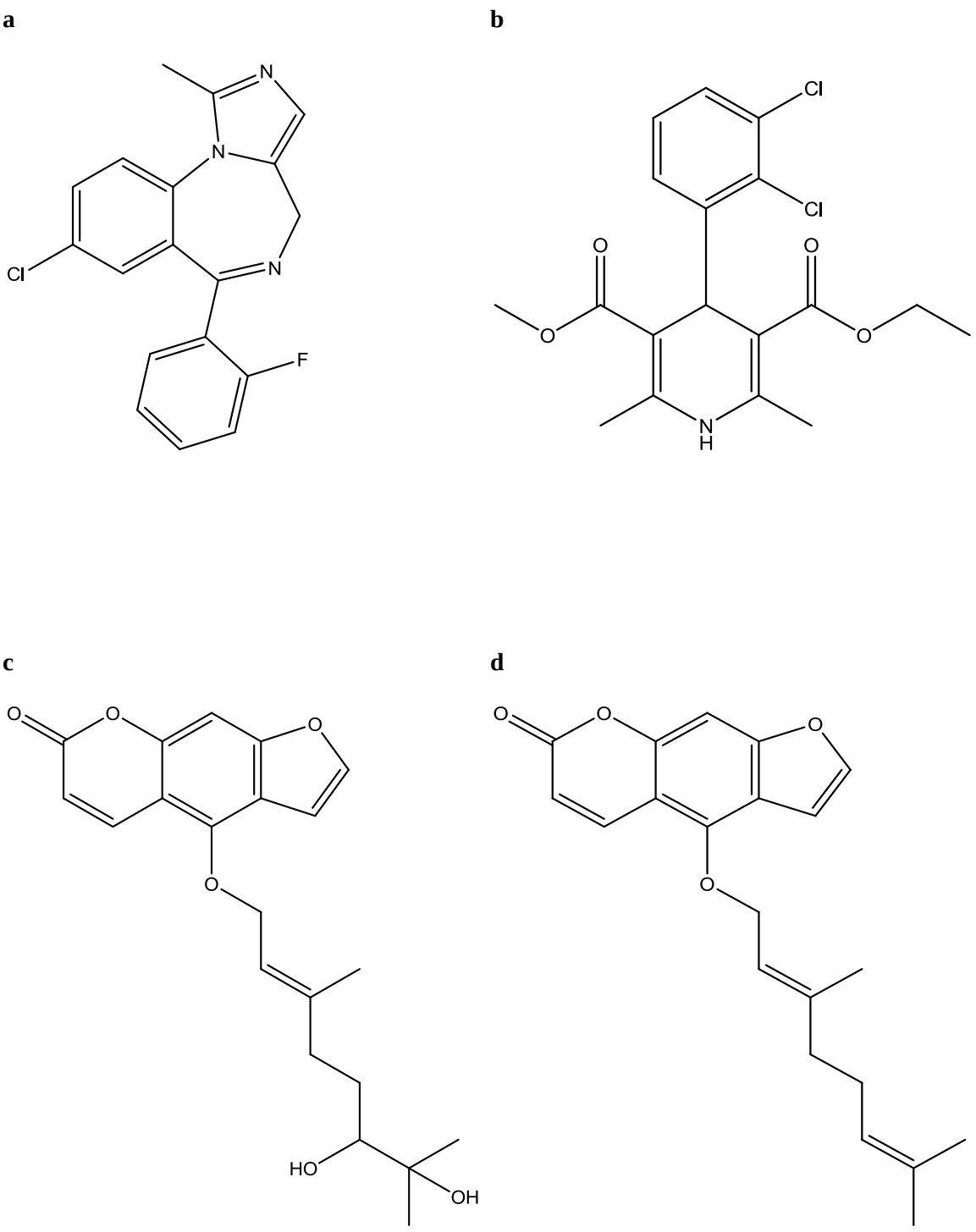
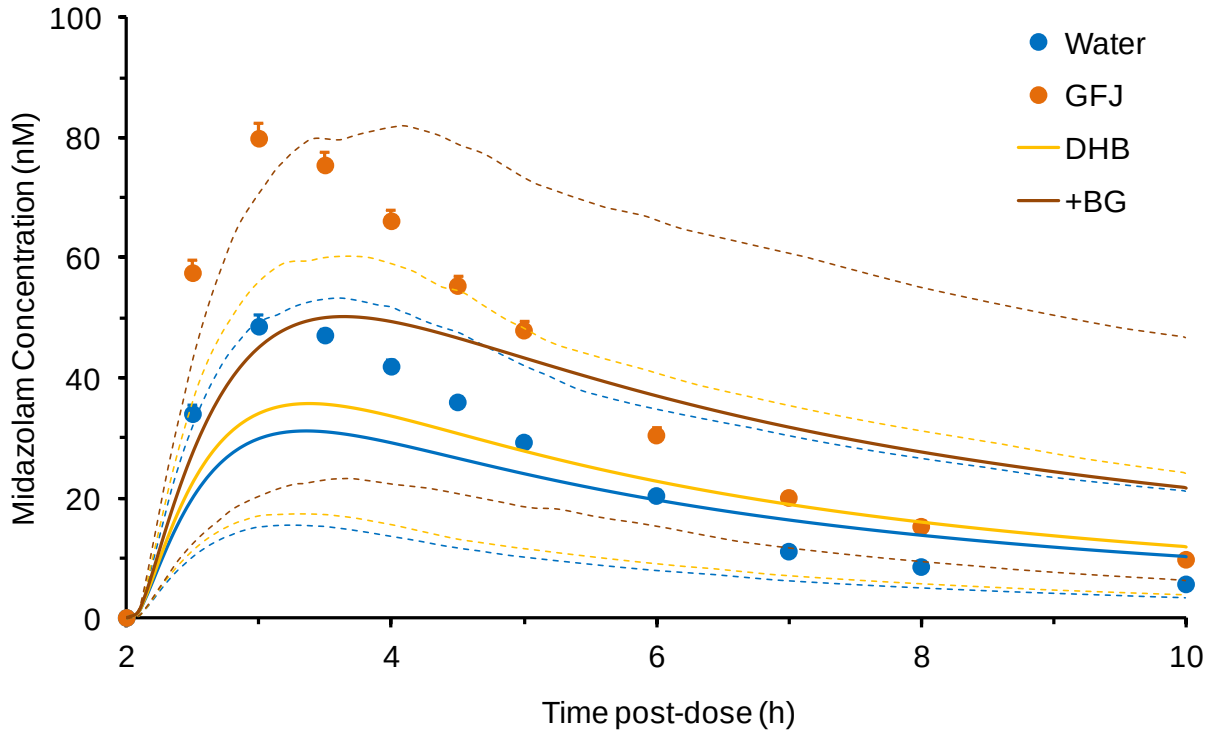


Fig. 4.1. Structures of midazolam (**a**), felodipine (**b**), 6',7'-dihydroxybergamottin (**c**), and bergamottin (**d**).

a


100
80
60
40
20
0
Midazolam Concentration (nM)
2
4
6
8
10
Time post-dose (h)
Water
GFJ
DHB
+BG

b

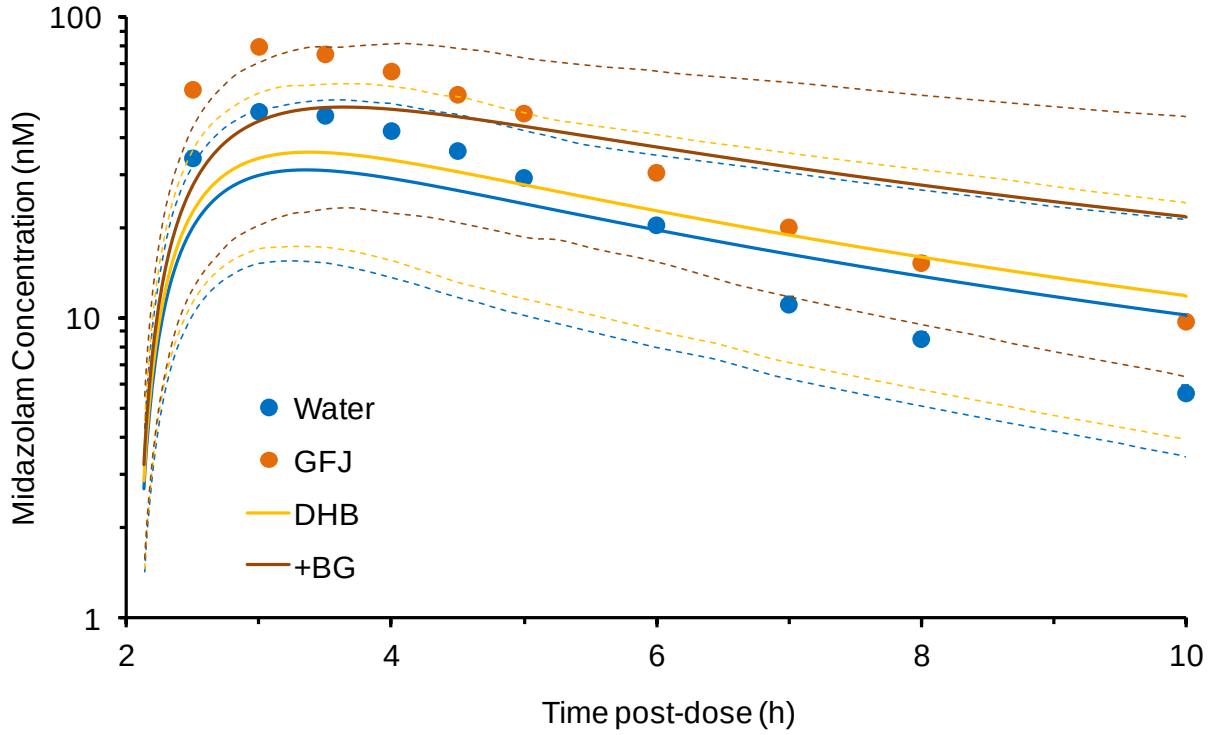
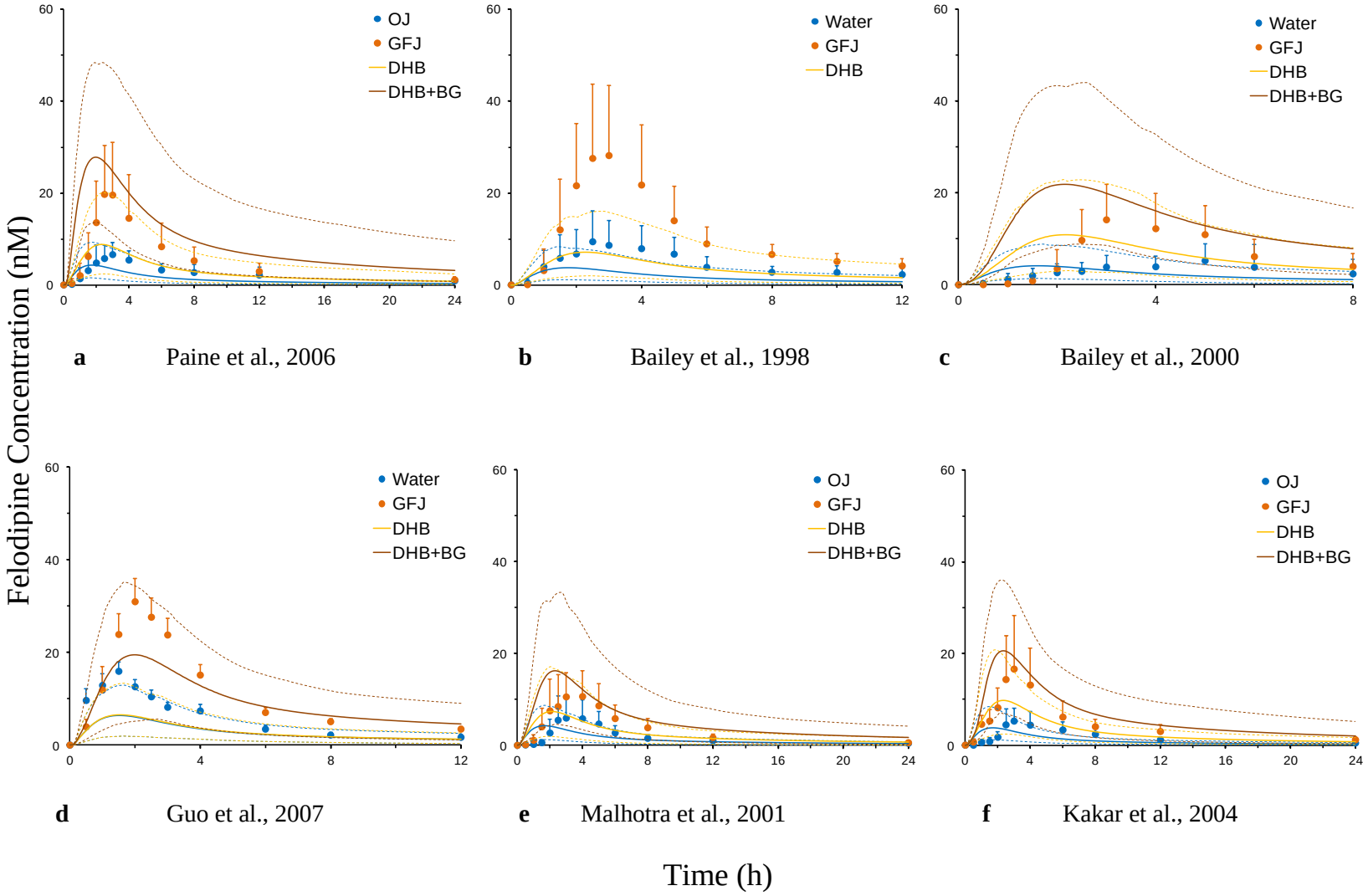

100
10
1
Midazolam Concentration (nM)
2
4
6
8
10
Time post-dose (h)
Water
GFJ
DHB
+BG

Fig. 4.2. Observed midazolam plasma concentration-time profile following administration of water (●) and GFJ (●) and simulated midazolam plasma concentration-time profiles following administration of DHB (—) and DHB plus BG (—) (**a**) and same profiles in semi-log scale (**b**). Data points denote observed mean values extracted from a published clinical study (13). Solid lines and dashed lines denote the mean profile and 90% confidence intervals of 10 trial simulations (n = 25 per trial, n = 250 total).


Felodipine Concentration (nM)
OJ
GFJ
DHB
DHB+BG
Water
a Paine et al., 2006
b Bailey et al., 1998
c Bailey et al., 2000
d Guo et al., 2007
e Malhotra et al., 2001
f Kakar et al., 2004
Time (h)

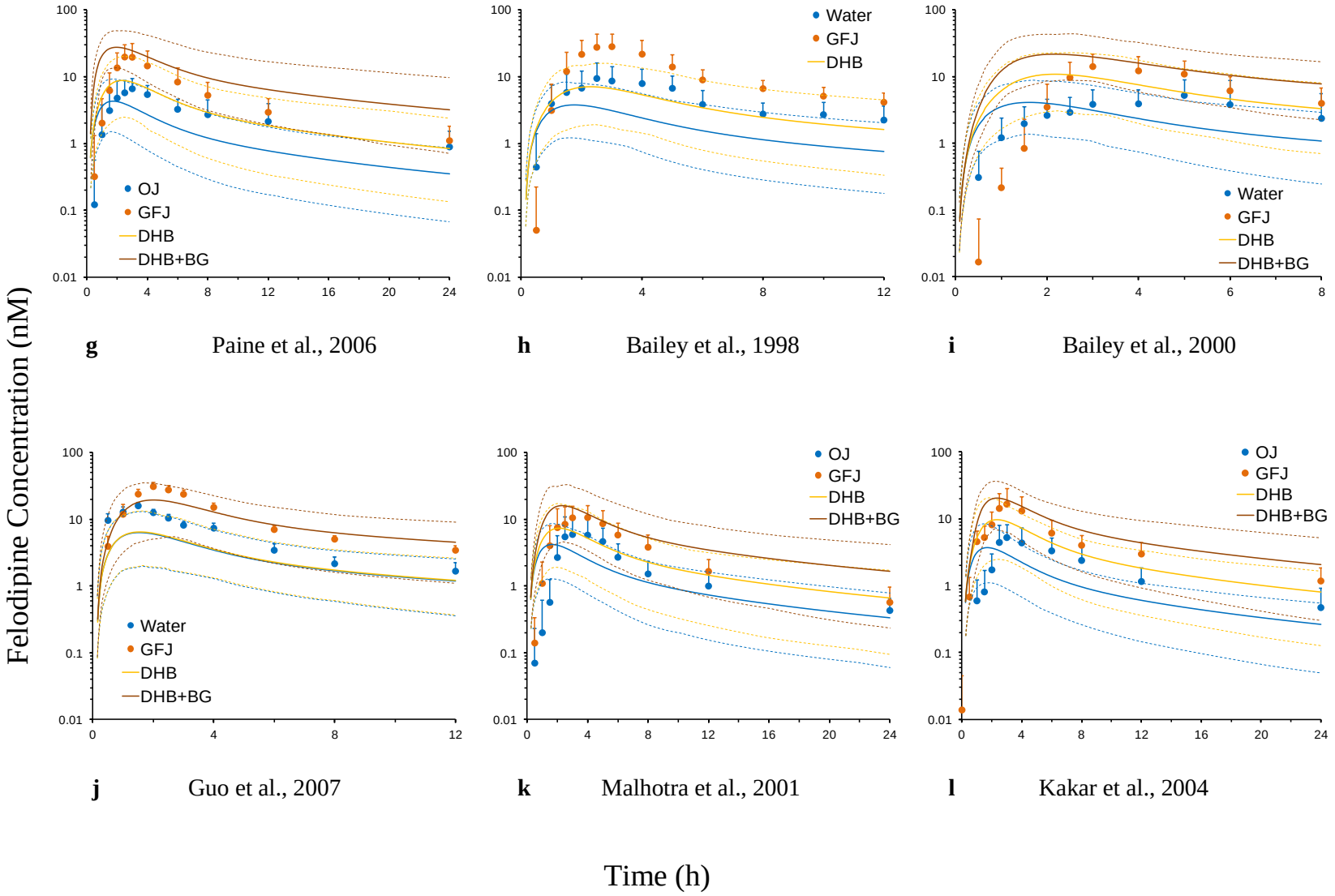
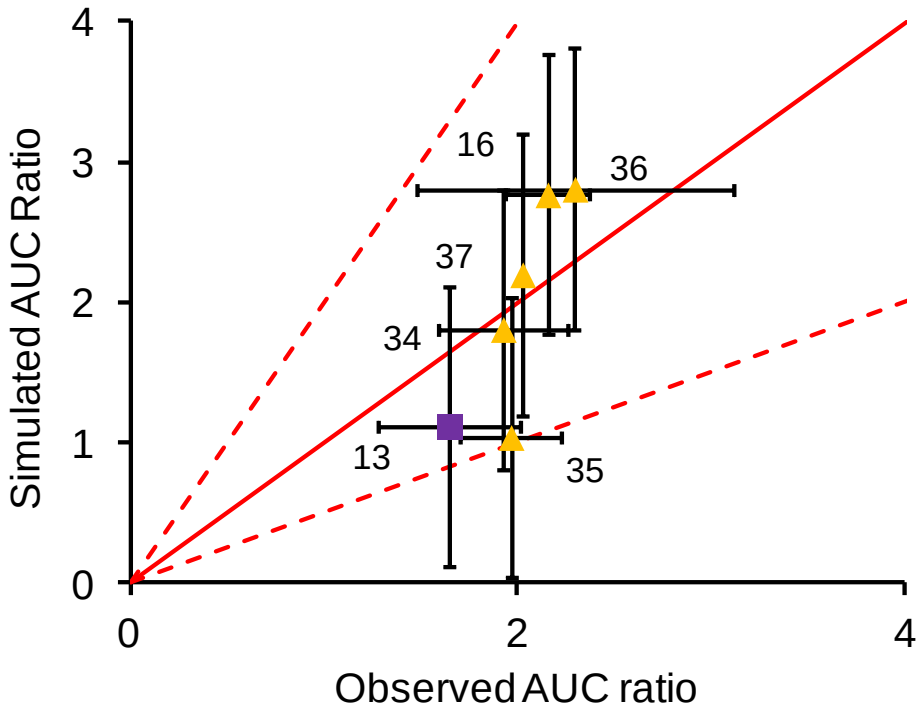

Felodipine Concentration (nM)
Time (h)
OJ
GFJ
DHB
DHB+BG
Water
g Paine et al., 2006
h Bailey et al., 1998
i Bailey et al., 2000
j Guo et al., 2007
k Malhotra et al., 2001
l Kakar et al., 2004

Fig. 4.3. Observed felodipine plasma concentration-time profile following administration of water or orange juice control (●) and GFJ (●) and simulated felodipine plasma concentration-time profile following administration of DHB (—) and DHB plus BG (—) (**a-f**) and same profiles in semi-log scale (**g-l**). Data points denote observed mean values extracted from published clinical studies. Solid lines and dashed lines denote the mean profile and 90% confidence intervals of 10 trial simulations (**a**, n = 180 total; **b**, **c**, **d**, n = 120 total each; **e**, n = 100 total; **f**, n = 50 total).

a


Simulated AUC Ratio
Observed AUC ratio
0
1
2
3
4
0
2
4
16
36
37
34
13
35

b

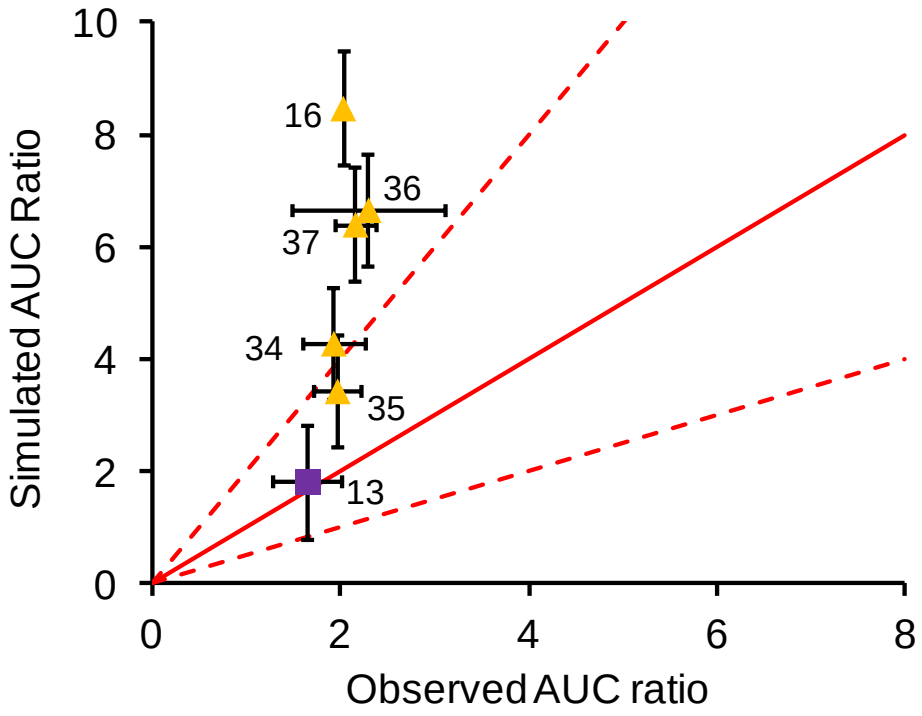

Simulated AUC Ratio
Observed AUC ratio
0
2
4
6
8
10
0
2
4
6
8
16
36
37
34
35
13

Fig. 4.4. Comparison of observed and simulated mean AUC ratios of selected clinical GFJ-drug interaction studies. DHB was given alone (**a**) then with BG (**b**) in the simulations. Symbols and denote mean AUC ratios. Horizontal and vertical error bars denote standard deviations of observed and simulated mean AUC ratios, respectively, of clinical studies (■, midazolam; ▲, felodipine). Numbers denote reference number. Solid line denotes line of unity. Dashed lines denote two-fold boundaries.

REFERENCES

[1] http://www.fda.gov/downloads/regulatoryinformation/guidances/ucm126833.pdf

[2] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003126.pdf

[3] Li Z, Vachharajani NN, Krishna R. On the assessment of effects of food on the pharmacokinetics of drugs in early development. Biopharm Drug Dispos. 2002;23(4):165-171.

[4] Custodio JM, Wu CY, Benet LZ. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. Adv Drug Deliv Rev. 2008;60(6):717-733.

[5] http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf

[6] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf

[7] Won CS, Oberlies NH, Paine MF. Influence of dietary substances on intestinal drug metabolism and transport. Curr Drug Metab. 2010;11(9):778-792.

[8] Bailey DG, Spence JD, Munoz C, Arnold JM. Interaction of citrus juices with felodipine and nifedipine. Lancet. 1991; 337(8736):268-269.

[9] Hanley MJ, Cancalon P, Widmer WW, Greenblatt DJ. The effect of grapefruit juice on drug disposition. Expert Opin Drug Metab Toxicol. 2011;7(3):267-286.

[10] Paine MF, Oberlies NH. Clinical relevance of the small intestine as an organ of drug elimination: drug-fruit juice interactions. Expert Opin Drug Metab Toxicol. 2007;3(1):67-80.

[11] Bailey DG, Dresser GK. Interactions between grapefruit juice and cardiovascular drugs. Am J Cardiovasc Drugs. 2004;4(5):281-297.

[12] Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, *et al*. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. J Clin Invest. 1997;99(10):2545-2553.

[13] Takanaga H, Ohnishi A, Matsuo H, Murakami H, Sata H, Kuroda K, *et al*. Pharmacokinetic analysis of felodipine-grapefruit juice interaction based on an irreversible enzyme inhibition model. Br J Clin Pharmacol. 2000;49(1):49-58.

[14] Greenblatt DJ, von Moltke LL, Harmatz JS, Chen G, Weemhoff JL, Jen C, *et al*. Time course of recovery of cytochrome P450 3A function after single doses of grapefruit juice. Clin Pharmacol Ther. 2003;74(2):121-129.

[15] Paine MF, Widmer WW, Hart HL, Pusek SN, Beavers KL, Criss AB, *et al*. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. Am J Clin Nutr. 2006;83(5):1097-1105.

[16] Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME, He K, Lown KS, Woster PM, *et al*. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. Drug Metab Dispos. 1997;25(11):1228-1233.

[17] He K, Iyer KR, Hayes RN, Sinz MW, Woolf TF, Hollenberg PF. Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. Chem Res Toxicol. 1998;11(4):252-259.

[18] Guo LQ, Fukuda K, Ohta T, Yamazoe Y. Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity. Drug Metab Dispos. 2000;28(7):766-771.

[19] Tassaneeyakul W, Guo LQ, Fukuda K, Ohta T, Yamazoe Y. Inhibition selectivity of grapefruit juice components on human cytochromes P450. Arch Biochem Biophys. 2000 Jun 15;378(2):356-363.

[20] Paine MF, Criss AB, Watkins PB. Two major grapefruit juice components differ in time to onset of intestinal CYP3A4 inhibition. J Pharmacol Exp Ther. 2005;312(3):1151-1160.

[21] De Castro WV, Mertens-Talcott S, Rubner A, Butterweck V, Derendorf H. Variation of flavonoids and furanocoumarins in grapefruit juices: a potential source of variability in grapefruit juice-drug interaction studies. J Agric Food Chem. 2006;54(1):249-255.

[22] Greenblatt DJ, Zhao Y, Hanley MJ, Chen C, Harmatz JS, Cancalon PF, *et al*. Mechanism-based inhibition of human cytochrome P450-3A activity by grapefruit hybrids having low furanocoumarin content. Xenobiotica. 2012 Jul 18. Doi:10.3109/00498254.2012.700428.

[23] Uesawa Y, Abe M, Fukuda E, Baba M, Okada Y, Mohri K. Construction of a model to estimate the CYP3A inhibitory effect of grapefruit juice. Pharmazie. 2011;66(7):525-528.

[24] Takanaga H, Ohnishi A, Murakami H, Matsuo H, Higuchi S, Urae A, *et al*. Relationship between time after intake of grapefruit juice and the effect on pharmacokinetics and pharmacodynamics of nisoldipine in healthy subjects. Clin Pharmacol Ther. 2000;67(3):201-214.

[25] Fenneteau F, Poulin P, Nekka F. Physiologically based predictions of the impact of inhibition of intestinal and hepatic metabolism on human pharmacokinetics of CYP3A substrates. J Pharm Sci. 2010;99(1):486-514.

[26] Zhao P, Zhang L, Grillo JA, Liu Q, Bullock JM, Moon YJ, et al. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. Clin Pharmacol Ther. 2011;89(2):259-267.

[27] Rowland M, Peck C, Tucker G. Physiologically-based pharmacokinetics in drug development and regulatory science. Annu Rev Pharmacol Toxicol. 2011;51(1):45-73.

[28] Huang SM, Rowland M. The role of physiologically based pharmacokinetic modeling in regulatory review. Clin Pharmacol Ther. 2012;91(3):542-549.

[29] Jamei M, Marciniak S, Feng K, Barnett A, Tucker G, Rostami-Hodjegan A. The Simcyp population-based ADME simulator. Expert Opin Drug Metab Toxicol. 2009:5(2):211-223.

[30] Parrott N, Lave T. Applications of physiologically based absorption models in drug discovery and development. Mol Pharm. 2008;5(5):760-775.

[31] Waldmann S, Almukainzi M, Bou-Chacra NA, Amidon GL, Lee BJ, Feng J, Kanfer I, Zuo JZ, Wei H, Bolger MB, Löbenberg R. Provisional biopharmaceutical classification of some common herbs used in Western medicine. Mol Pharm. 2012;9(4):815-822.

[32] Goosen TC, Cillie D, Bailey DG, Yu C, He K, Hollenberg PF, *et al*. Bergamottin contribution to the grapefruit juice-felodipine interaction and disposition in humans. Clin Pharmacol Ther. 2004;76(6):607-617.

[33] Uesawa Y, Yamada H, Mohri K. Determination of bergamottin in human plasma after grapefruit juice ingestion by an UPLC/MS/MS method. Pharmazie. 2008;63(2):110-112.

[34] Bailey DG, Kreeft JH, Munoz C, Freeman DJ, Bend JR. Grapefruit juice-felodipine interaction: effect of naringin and 6',7'-dihydroxybergamottin in humans. Clin Pharmacol Ther. 1998;64(3):248-256.

[35] Bailey DG, Kreeft JH, Munoz C, Freeman DJ, Bend JR. Grapefruit-felodipine interaction: effect of unprocessed fruit and probable active ingredients. Clin Pharmacol Ther. 2000;68(5):468-477.

[36] Guo LQ, Chen QY, Wang X, Liu YX, Chu XM, Cao XM, Li JH, Yamazoe Y. Different roles of pummelo furanocoumarin and cytochrome P450 3A5*3 polymorphism in the fate and action of felodipine. Curr Drug Metab. 2007;8(6):623-630.

[37] Malhotra S, Bailey DG, Paine MF, Watkins PB. Seville orange juice-felodipine interaction:

comparison with dilute grapefruit juice and involvement of furocoumarins. Clin Pharmacol Ther. 2001;69(1):14-23.

[38] Kakar SM, Paine MF, Stewart PW, Watkins PB. 6'7'-Dihydroxybergamottin contributes to the grapefruit juice effect. Clin Pharmacol Ther. 2004;75(6):569-579.

[39] Gertz M, Harrison A, Houston JB, Galetin A. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. Drug Metab Dispos. 2010;38(7):1147-1158.

[40] Gertz M, Houston JB, Galetin A. Physiologically based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction. Drug Metab Dispos. 2011;39(9):1633-1642.

[41] Valle M, Esteban M, Rodríguez-Sasiaín JM, Calvo R, Aguirre C. Characteristics of serum protein binding of felodipine. Res Commun Mol Pathol Pharmacol. 1996;94(1):73-88.

[42] Kochansky CJ, McMasters DR, Lu P, Koeplinger KA, Kerr HH, Shou M, Korzekwa KR. Impact of pH on plasma protein binding in equilibrium dialysis. Mol Pharm. 2008;5(3):438-448.

[43] Yang J, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A. Prediction of intestinal first-pass drug metabolism. Curr Drug Metab. 2007;8(7):676-684.

[44] Edgar B, Regårdh CG, Johnsson G, Johansson L, Lundborg P, Löfberg I, Rönn O. Felodipine kinetics in healthy men. Clin Pharmacol Ther. 1985;38(2):205-211.

[45] Hou TJ, Zhang W, Xia K, Qiao XB, Xu XJ. ADME evaluation in drug discovery. 5. Correlation of Caco-2 permeation with simple molecular properties. J Chem Inf Comput Sci. 2004;44(5):1585-1600.

[46] Rodgers T, Leahy D, Rowland M. Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. J Pharm Sci. 2005;94(6):1259-1276.

[47] Rodgers T, Rowland M. Physiologically based pharmacokinetic 200odeling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci. 2006 Jun;95(6):1238-1257.

[48] Walsky RL, Obach RS. Validated assays for human cytochrome P450 activities. Drug Metab Dispos. 2004;32(6):647-660.

[49] Paine MF, Criss AB, Watkins PB. Two major grapefruit juice components differ in intestinal

CYP3A4 inhibition kinetic and binding properties. Drug Metab Dispos. 2004;32(10):1146-1153.

[50] Bailey DG, Dresser GK, Bend JR. Bergamottin, lime juice, and red wine as inhibitors of cytochrome P450 3A4 activity: comparison with grapefruit juice. Clin Pharmacol Ther. 2003;73(6):529-537.

[51] Brown J, Crison J, Timmins P. Predicting feasibility and characterizing performance of extended-release formulations using physiologically based pharmacokinetic modeling. Ther Deliv. 2012;3(9):1047-1059.

[52] Row E, Brown SA, Stachulski AV, Lennard MS. Development of novel furanocoumarin dimers
201 as potent and selective inhibitors of CYP3A4. Drug Metab Dispos. 2006;34(2):324-30.

[53] Galetin A, Clarke SE, Houston JB. Multisite kinetic analysis of interactions between prototypical CYP3A4 subgroup substrates: midazolam, testosterone, and nifedipine. Drug Metab Dispos. 2003;31(9):1108-1116.

[54] Guest EJ, Rowland-Yeo K, Rostami-Hodjegan A, Tucker GT, Houston JB, Galetin A. Assessment of algorithms for predicting drug-drug interactions via inhibition mechanisms: comparison of dynamic and static models. Br J Clin Pharmacol. 2011;71(1):72-87.

[55] Lin HL, Kenaan C, Hollenberg PF. Identification of the residue in human CYP3A4 that is covalently modified by bergamottin and the reactive intermediate that contributes to the grapefruit juice effect. Drug Metab Dispos. 2012;40(5):998-1006.

[56] Messer A, Nieborowski A, Strasser C, Lohr C, Schrenk D. Major furocoumarins in grapefruit juice I: levels and urinary metabolite(s). Food Chem Toxicol. 2011;49(12):3224-3231.

[57] Ayan-Oshodi MA, Willis BA, Annes WF, Lowe SL, Friedrich S, de la Peña A, *et al*. The effects on metabolic clearance when administering a potent CYP3A autoinducer with the prototypic CYP3A inhibitor, ketoconazole. Drug Metab Dispos. 2012;40(10):1945-1952.

[58] Wojnowski L, Kamdem LK. Clinical implications of CYP3A polymorphisms. Expert Opin Drug Metab Toxicol. 2006;2(2):171-182.

[59] Kimura S, Kako S, Wada H, Sakamoto K, Ashizawa M, Sato M, *et al*. Can grapefruit juice decrease the cost of imatinib for the treatment of chronic myelogenous leukemia? Leuk Res. 2011;35(1):e11-2.

[60] Reif S, Nicolson MC, Bisset D, Reid M, Kloft C, Jaehde U, *et al*. Effect of grapefruit juice intake on etoposide bioavailability. Eur J Clin Pharmacol. 2002;58(7):491-4.

[61] Liu C, Shang YF, Zhang XF, Zhang XG, Wang B, Wu Z, *et al*. Co-administration of grapefruit juice increases bioavailability of tacrolimus in liver transplant patients: a prospective study. Eur J Clin Pharmacol. 2009;65(9):881-5.

[62] Cohen EE, Wu K, Hartford C, Kocherginsky M, Eaton KN, Zha Y, *et al*. Phase I studies of sirolimus alone or in combination with pharmacokinetic modulators in advanced cancer patients. Clin Cancer Res. 2012;18(17):4785-93.

CHAPTER 5

CONCLUSIONS

The daily act of consuming food is integral to how the body responds to foreign substances such as environmental toxins and pharmacologic agents. The activity of drugs and dietary substances can be linked, as they share the same processes of absorption, distribution, metabolism, and excretion [1]. Since most of the drugs used in pharmacotherapy are administered orally, the bioavailability and, ultimately, efficacy and safety of these compounds can be influenced by transport and metabolism in the intestine, a critical barrier that must be overcome. Fruit juices, an extensively marketed food product, have been shown to contain constituents that modulate intestinal drug metabolizing enzymes (*e.g.*, cytochrome P450s) and transport proteins (*e.g.*, organic anion transporting polypeptides), leading to altered pharmacokinetics of victim drugs. Fruit juice-drug interactions have become increasingly recognized for both new and established medicines. Thus, improvements, and eventual standards, in experimental design and interpretation of such interaction studies are needed to evaluate clinical relevance. Although robust *in vitro* and *in vivo* methods (such as those used in drug development) are available to screen for interaction liability and to elucidate underlying mechanisms, cited interactions (or lack thereof) purported to occur are of questionable validity, as highly variable components can contribute to various magnitudes of effect on multiple drugs. Such a "one-brand-fits-all" approach is inappropriate, as complex bioactive ingredient composition undoubtedly contributes to the lack of replication and discrepancies between in *vitro* predictions and *in vivo* observations.

The overall goal of this dissertation project was to develop a framework for how to conduct research on dietary substance-drug interactions using *in vitro*, *in vivo*, and *in silico* methods. These three approaches were discussed in the context of grapefruit juice (GFJ), which was used as a model

dietary substance. Although GFJ has been studied for more than 20 years, new insights continue to be uncovered. The majority of GFJ-drug interactions to date have focused on those mediated by intestinal cytochrome P450 3A4 (CYP3A4), but a more recently discovered mechanism involving inhibition of organic anion-transporting polypeptides (OATPs) has been identified for several clinically important drugs [2]. *In vitro* and *in vivo* methods were applied to the study of this mechanism. The 'GFJ effect' mediated by CYP3A4 was revisited using *in silico* methods. Major observations, novelties, limitations, and future studies of each application are discussed.

***In Vivo*: Evaluation of Furanocoumarins and Polymethoxyflavones as Major Mediators of the GFJ-Fexofenadine Interaction in Healthy Volunteers**

Evaluation of the inhibitory effects of specific juice components on intestinal OATP activity *in vivo* can pose a challenge in the clinical setting. Although specific constituents can be administered as a single phytochemical entity [3] or mixture of select entities, one or a few candidate(s) is(are) unlikely to explain fully the collective activity of hundreds of other potential inhibitors. Select GFJ flavonoids (naringin, naringenin, hesperidin, hesperetin, quercetin, tangeretin, nobiletin) and furanocoumarins (6',7'-dihydroxybergamottin, bergamottin) have been identified as OATP inhibitors *in vitro* [3-5]. Only naringin, given as a single entity in aqueous solution form, has been shown to contribute, in part, to the interaction *in vivo*. Taken together, the causative ingredients mediating the OATP-mediated GFJ-fexofenadine interaction in humans were investigated in Chapter 2.

A food-grade GFJ devoid (> 97-99%) of furanocoumarins and polymethoxyflavones (mGFJ) was used to evaluate these compounds as inhibitors of enteric OATP-mediated uptake of fexofenadine in 18 healthy volunteers and OATP-transfected cells. Compared to water, both mGFJ and GFJ decreased fexofenadine geometric mean AUC and C_{max}, by ~25%, with no effect on terminal half-life. Furanocoumarins and polymethoxyflavones have been identified as OATP inhibitors *in vitro* [3, 4]. Similar effects by both juices on fexofenadine pharmacokinetics indicated that furanocoumarins and polymethoxyflavones are not major mediators of the GFJ-fexofenadine interaction *in vivo*. The GFJ used in this *in vivo* approach provided a unique tool that allowed

assessment of particular GFJ compounds in aggregate. The clinical study was designed to address common limitations encountered in most fruit juice-drug interaction studies: relevant/suspected bioactive constituent(s) not measured by a validated analytical method, sample size not justified by a power calculation, blood sampling times not appropriate, and/or a dosing regimen not reflective of 'real world' scenarios. The effects of mGFJ and GFJ extracts on OATP1A2 and OATP2B1 uptake activity were compared in transfected cells using estrone 3-sulfate and fexofenadine as probe substrates. If possible, *in vitro* experiments mirroring the clinical situation should be conducted before initiating a clinical study. Such investigations can provide predictive and mechanistic insight. Preliminary *in vitro* studies with estrone 3-sulfate mimicking clinical conditions showed similar OATP1A2/2B1 inhibitory behavior between both juices (~50% inhibition *vs*. vehicle). Follow-up *in vitro* experiments with fexofenadine mimicking the clinical setting showed similar extents of OATP1A2 (but not OATP2B1) inhibition by both juices (~25%) compared to that observed in healthy volunteers (~25%). The *in vitro* and *in vivo* results agreed in this case, but even if they had not, at least the aforementioned study limitations could be eliminated as possible confounding factors.

The following future directions are recommended based on the implications of these studies:

a. Results of the GFJ-mGFJ-fexofenadine clinical study suggest other flavonoids as enteric OATP inhibitors. Naringin, naringenin, quercetin, and hesperidin have been shown to inhibit intestinal OATP activity *in vitro* [3, 4]. Only naringin has been shown to contribute (partly) to the GFJ-fexofenadine interaction *in vivo* [3]. GFJ contains other flavonoids (*e.g.*, narirutin, didymin, poncirin) [6] that may have inhibitory potential toward OATPs but remain unexplored *in vitro* and *in vivo*. The effect of a GFJ devoid of flavonoids or a mixture of select purified flavonoids in various combinations (prepared in concentrations equivalent to that present in selected GFJ) on fexofenadine could be compared in healthy volunteers. Screening *in vitro* inhibition studies and analysis of flavonoid content in GFJs should be conducted before and/or during clinical investigations.

b. Although fexofenadine was used as an OATP probe substrate in the current study, the *in vivo* effect of furanocoumarins, polymethoxyflavones, and other flavonoids on other currently marketed OATP substrates (*e.g.*, celiprolol, talinolol, atenolol, levofloxacin, ciprofloxacin, aliskiren, statins) has not been investigated in healthy (or diseased) populations. For some of the drugs, clinical GFJ interaction studies have not been conducted or reproduced; reported studies have involved orange juice. After establishing a clinical pharmacokinetic effect of whole GFJ in humans, a similar approach described in Chapter 2 for the aforementioned drugs could be applied to identify causative ingredients.

c. The mGFJ has potential utility as a tool to discern the effects of causative ingredients on drug disposition. The mGFJ has been used to investigate other mechanisms underlying the 'GFJ effect' (*i.e.*, CYP3A4-mediated, P-gp-mediated) [7, 8]. GFJ has been shown to inhibit other drug metabolizing enzymes *in vitro* and in rats such as esterases [9, 10] and sulfotransferases [11, 12]. The mGFJ could be used to evaluate the causative ingredients of these non-CYP-mediated mechanisms if a clinically relevant drug has been identified (*e.g.*, esterase – lovastatin, enalapril, clopidogrel).

***In Vitro*: Bioactivity-Guided Fractionation of GFJ and Evaluation of Representative Components as Inhibitors of an Intestinal Organic Anion-Transporting Polypeptide**

Despite the abundance of knowledge on GFJ amassed over two decades, the various components that comprise this beverage continue to reveal new information, demonstrating effects on critical mechanisms needed to eliminate drugs from the body. The GFJ-mediated decrease in drug absorption *via* inhibition of intestinal organic anion transporting polypeptides (OATPs) is one of the most widely studied transporter-based dietary substance-drug interactions. However, only a handful of GFJ constituents capable of inhibiting OATP have been assessed *in vitro* (and even less *in vivo*). In Chapter 2, other flavonoids retained in the mGFJ represent candidate enteric OATP inhibitors. However, hundreds of compounds are in this class. The difficulty in identifying inhibitors arises from the complex composition of GFJ. The "cherry-picking" approach to studying individual causative

ingredients contributed to the >15 years elapsed to establish furanocoumarins, in aggregate, as CYP3A inhibitors *in vivo* [7]. The *in vitro* studies in Chapter 3 were designed to streamline the process. A method routinely used in the natural products field, bioactivity-guided isolation, was used to identify, systematically, OATP inhibitors in GFJ. Organic-soluble fractions generated from an extract of a commercially available GFJ product were evaluated as inhibitors of OATP2B1-mediated uptake using stably transfected Madin-Darby canine kidney type II (MDCKII) cells and estrone 3-sulfate as the probe substrate. Advanced rounds of fractionation of active pools and subsequent complete structure characterization were not possible due to inadequatematerial. A starting volume of *>100 L* of the *same* lot of grapefruit juice would have been sufficient but was impractical to obtain. Such a limitation demonstrated that juice is not ideal material for fractionation due to high content of water, sugar, and preservatives, as well as possible contamination with juices of other fruits [13]. As a consequence, dried whole grapefruit should be used in future studies, as such a material is less cumbersome analytically and can be vouchered, permitting verification (a common problem in natural products research) of the identity of the specific plant used in a study. Nevertheless, the *in vitro* approach taken in Chapter 3 was, to the author's knowledge, the first to fractionate juice material (GFJ in this case) to identify intestinal OATP inhibitors (OATP2B1 in particular) using an overexpressing cell system. A similar approach was taken to identify CYP3A inhibitors in cranberry and liver OATP1B1/1B3 inhibitors in *Rollinia emarginata* [13, 14]. These examples illustrate that bioactivity-guided isolation assays can be designed according to the mechanism, appropriate cell system, and probe(s) of interest. Given the lack of individual isolated compounds from the fractionation procedure, known GFJ constituents were evaluated for OATP2B1 inhibitory potency *in vitro*. This assessment should be routine in combination with bioactivity-guided isolation. The ultimate goal of this isolation procedure is to 1) remove inhibitory ingredients (similar to mGFJ) to test clinically, 2) select individual/combinations of purified ingredients to test clinically, and/or 3) discover new juice manufacturing techniques (heat, microbial additives, plant engineering) to produce low amounts of one or more ingredients [15].

Additional future directions are recommended as a follow-up to these studies:

a. Although estrone 3-sulfate was used as the probe substrate in the isolation and potency assays, other, clinically relevant drugs could be tested (*e.g.*, celiprolol, talinolol, atenolol, levofloxacin, ciprofloxacin, aliskiren, some statins), as a particular OATP substrate may yield a different set of OATP inhibitors. Although OATP2B1 was the focus of the assays in Chapter 3, OATP1A2 is another intestinal OATP of emerging interest. The role of OATP1A2 in the transport of the aforementioned drugs has not been elucidated fully. Thus, for a given OATP substrate, the set of inhibitors for OATP1A2 versus OATP2B1 may be different.

b. Orange and apple juice have been shown to inhibit intestinal OATP *in vitro* and *in vivo* [16]. Although candidate causative ingredients in orange (hesperidin) and apple (phloridzin, phloretin) juices have been identified, the list is not comprehensive. Other juices studied as potential perpetrators of drug interactions include cranberry and pomegranate juices (Appendix B and C), which have not been evaluated as OATP inhibitors *in vivo*. Bioactivity-guided isolation could be used to identify intestinal OATP inhibitors in these non-grapefruit juices.

c. Although the "GFJ effect" (initially defined as CYP3A-mediated interactions) has been expanded over the years to include esterases, sulfotransferases, P-glycoprotein, OATP, and multidrug resistance-associated protein (MRP) 2, other mechanisms such as those mediated by intestinal breast cancer resistance protein (BCRP), organic solute transporter (OST) α/β, and other MRPs could be evaluated. Due to the availability of stably transfected systems expressing the aforementioned transporters, the effect of GFJ and individual compounds can be examined *in vitro*.

***In Silico*: Evaluation of Select GFJ Constituents as Marker Compounds Predictive of the GFJ Effect**

Dietary substances are complex mixtures of multiple bioactive ingredients and may not always produce similar, reproducible results. An increased understanding of the causative bioactive

components and pharmacokinetic mechanisms is required to provide firm recommendations on how to manage food-drug interactions. The first step is rigorous *in vitro* characterization of a given dietary substance prior to conducting statistically powered human studies designed to evaluate drug interaction liability. Ultimately, the *in vivo* scenario is of the greatest concern. How does this leap from *in vitro* data to *in vivo* evaluation occur? *In silico* tools can provide an early, as well as retrospective, assessment of drug interaction potential based on *in vitro* and *in vivo* data. Several physiologically-based pharmacokinetic (PBPK) models, including a description of intestinal processes, have been applied for predicting oral pharmacokinetics [17]. The commercially available software platform Simcyp® was used to evaluate the furanocoumarins 6',7'-dihydroxybergamottin (DHB) and bergamottin as candidate marker substances predictive of CYP3A4-mediated GFJ-drug interactions. *In vitro*, *in vivo*, and *in silico*-derived parameters describing absorption, distribution, metabolism, and excretion properties of the model oral CYP3A4 substrates midazolam and felodipine and the candidate furanocoumarins were collected from literature and implemented in Simcyp®. DHB alone was predictive of midazolam and felodipine mean AUC ratios (within two-fold) compared to observed clinical data. The addition of bergamottin overpredicted felodipine mean AUC ratios (two- to four-fold), highlighting a deficiency in the understanding of bergamottin behavior. Nevertheless, the *in silico* approach taken in Chapter 4 to evaluate GFJ ingredient-drug interactions is one of the few to take GFJ ingredient composition into account and the first to incorporate actual, realistic amounts of DHB and bergamottin intake into a PBPK interaction model [18-20].

A number of future directions are recommended by the results of these studies:

a. Enzyme kinetics describing metabolism of DHB and bergamottin were estimated from software and sparse clinical data. The K_m and V_{max} of DHB and BG should be determined experimentally to improve accuracy of the model.
b. Inhibitory kinetics of DHB and bergamottin specific to felodipine should be determined experimentally in human intestinal microsomes and recombinant CYP3A4.

c. The effect of DHB and bergamottin has not been elucidated in CYP3A5 expressers *in vitro* and *in vivo*. *In vitro* studies with DHB and bergamottin using CYP3A5-expressing human intestinal microsomes and recombinant CYP3A5 could be performed. The effect of DHB, bergamottin, and/or whole GFJ could be evaluated in humans who have been genotyped for CYP3A5 variant alleles (*e.g.*, *CYP3A5*3*).

d. If sufficient *in vitro* and *in vivo* data become available, other potent furanocoumarins (*e.g.*, furanocoumarin dimers) could be evaluated as other GFJ effect predictors.

e. Given the abundance of other GFJ-CYP3A substrate interactions studied clinically, retrospective analysis using the validated model could be performed. For example, the Simcyp® substrate library contains a number of CYP3A substrates. Once DHB and bergamottin inhibitor files are confirmed, a virtual trial could be conducted. One drawback is that most clinical studies did not report the concentration of DHB and/or bergamottin in the test GFJ. Because the range of concentrations in commercial GFJs is known, it may be possible to input a range of DHB and/or bergamottin "doses", compare the substrate exposures, and back-calculate a possible concentration (range) in the clinical test juice.

f. Assuming sufficient *in vitro* and *in vivo* data have been collected for a previously unassessed CYP3A substrate, a potential GFJ-CYP3A substrate interaction could be prospectively predicted and inform anticipated clinical studies.

g. Predicting the impact of GFJ on dual CYP3A/P-gp and P-gp/OATP or OATP substrates (*e.g.*, talinolol, cyclosporine, loperamide) could be evaluated using modeling and simulation.

EPILOGUE

A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease is defined as a drug and subjected to a battery of regulatory reviews. Natural products continue to provide a basis for health care and medicine to approximately 80% of the world population [21], even though the scientific evidence supporting their use has been weak, scant, or nonexistent. Today's myriad foods are laden with "wellness aids" (*i.e.*, the latest touted superfoods) or marketed heavily to reduce the risk of disease. Some food ingredients have been formulated as solid dosage forms and are sold alongside conventional over-the-counter medications, leading to misconceptions that these dietary supplements are therapeutic substitutes. Regular consumption of botanically-derived foods and supplements has fueled a global, multibillion-dollar industry, which is largely unregulated. The majority of phytochemicals is untested, and, coupled with the steadily increasing trend of polypharmacy, the population is at risk for dietary substance-drug interactions. Given the conservative nature of the current scientific and economic climate, efficient and robust research methodologies are needed to sustain the rise in consumer demand for these products and clinicians' concerns for patient safety monitoring.

This dissertation project specifically addressed fruit juices as perpetrators of dietary substance-drug interactions. The critical confounding factor of constituent composition in the context of interactions involving intestinal OATPs and CYP3A was the focus. The global objective of this research was to develop a framework (and establish some basic, required guidelines) for how to conduct research on dietary substances so that ultimately, associated risks can be better predicted, assessed, and managed. Results also highlighted the challenges that face scientists when trying to predict dietary substance-drug interactions. The aforementioned framework involves the adaptation of some guidelines for drug-drug interaction investigations [22], integrating data obtained from *in vitro* systems, *in silico* models, and clinical studies in an integrated manner to predict prospectively (and retrospectively) interactions. The intent is to ensure the same degree of confidence in quality and clinical utility as for drugs [23] but recognizing and accounting for the unique composition of natural

products. Based on the lessons gleaned from this dissertation and in light of the existing literature, a draft summary of recommendations and strategies relevant to inhibition-based interactions is presented in Figure 5.1 and Table 5.1.

The importance of food-drug interactions has been gaining more attention, and researchers are trying to answer emerging concerns about the impact of food on drug metabolism/transport. Investigations of complex botanical products require collaboration from experts in botany, pharmacognosy/natural products chemistry, (clinical) pharmacology on the bench end and health care providers (*e.g.*, prescribers, pharmacists, dietitians) on the beside end [24]. A multidisciplinary, translational research approach is necessary to explain fully these relatively unexplored types of drug interactions.

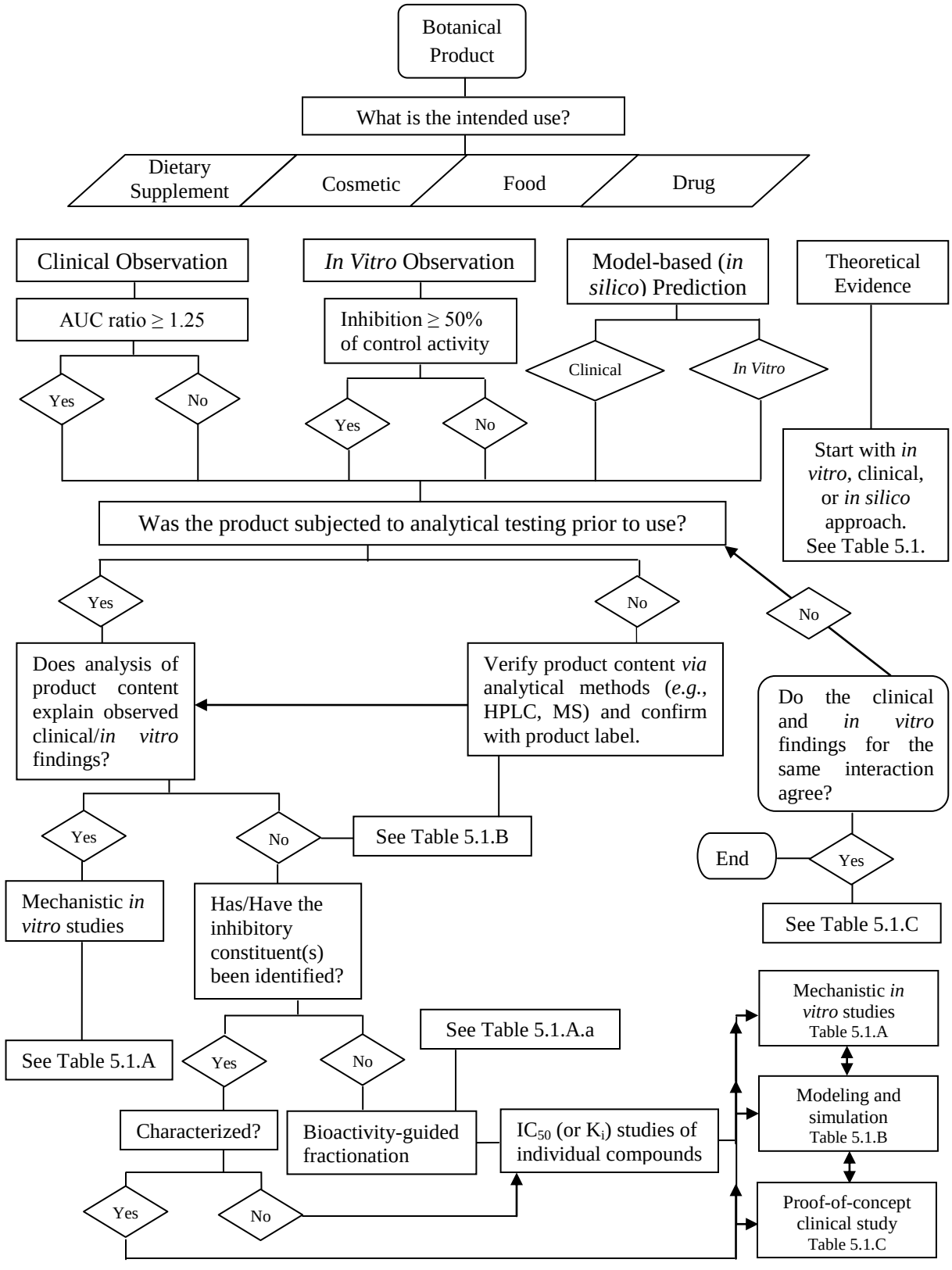


Figure 5.1. Considerations for the evaluation, interpretation, and conduct of natural product-drug interaction studies.

Table 5.1

Minimum recommended requirements for conducting *in vitro*, clinical, and *in silico* studies

For all studies, provide sufficient product information.

- Commercially available: brand name, manufacturer, lot number, ingredients, preparation directions, manufacturing process, origins of growth and production
- Freshly prepared: scientific name, quantity, plant part used, site of collection, preparation procedures, storage conditions

A. *In Vitro* Studies

1. Use relevant cell system(s) based on mechanism(s) of interest (*e.g.*, transfected cell line for transporters, pooled microsomes for metabolizing enzymes).
2. Choose appropriate probe substrate(s) and concentration.
3. Test negative and positive control inhibitors.

a. Bioactivity-guided fractionation
   1. Obtain ≥ 1 kg of plant material[†] or ≥ 100L of liquid material[‡] (*e.g.*, juice).
   2. Choose appropriate range of inhibitor concentrations.

B. Clinical Studies

1. Administer the same product brand and lot/batch to all subjects.
2. Measure any relevant/suspected bioactive constituent(s) *via* a validated analytical method (including custom-synthesized product/plant).
3. Justify sample size with power calculation.
   a. If pharmacogenetics is a variable, justify sample size.
   b. Randomize, blind (if possible), and administer a placebo.
4. Establish appropriate sampling times for substrate and inhibitor(s).
5. Impose dietary restrictions on the subjects during the study period.

C. *In Silico* Studies

1. Select appropriate program to execute model equations and perform simulations (*e.g.*, acslX, MATLAB-simulink, Berkeley-Madonna, GastroPlus, Simcyp).
2. Obtain observed plasma concentration-time profile(s).
3. Elucidate physicochemical properties of substrate and product constituents.

[†] vouchered specimen

[‡] same lot/batch

REFERENCES

[1] Raiten DJ. Nutrition and pharmacology: general principles and implications for HIV. Am J Clin Nutr. 2011;94:1697S-1702S.

[2] Dolton MJ, Roufogalis BD, McLachlan AJ. Fruit juices as perpetrators of drug interactions: the role of organic anion-transporting polypeptides. Clin Pharmacol Ther. 2012;92(5):622-30.

[3] Bailey DG, Dresser GK, Leake BF, Kim RB. Naringin is a major and selective clinical inhibitor of organic anion-transporting polypeptide 1A2 (OATP1A2) in grapefruit juice. Clin Pharmacol Ther. 2007;81(4):495-502.

[4] Satoh H, Yamashita F, Tsujimoto M, Murakami H, Koyabu N, Ohtani H, *et al*. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. Drug Metab Dispos. 2005;33(4):518-23.

[5] Shirasaka Y, Shichiri M, Mori T, Nakanishi T, Tamai I. Major active components in grapefruit, orange, and apple juices responsible for OATP2B1-mediated drug interactions. J Pharm Sci. 2013;102(1):280-8.

[6] Ross SA, Ziska DS, Zhao K, ElSohly MA. Variance of common flavonoids by brand of grapefruit juice. Fitoterapia. 2000;71(2):154-61.

[7] Paine MF, Widmer WW, Hart HL, Pusek SN, Beavers KL, Criss AB, *et al*. A furanocoumarin-free grapefruit juice establishes furanocoumarins as the mediators of the grapefruit juice-felodipine interaction. Am J Clin Nutr. 2006;83(5):1097-105.

[8] Paine MF, Widmer WW, Pusek SN, Beavers KL, Criss AB, Snyder J, *et al*. Further characterization of a furanocoumarin-free grapefruit juice on drug disposition: studies with cyclosporine. Am J Clin Nutr. 2008;87(4):863-71.

[9] Li P, Callery PS, Gan LS, Balani SK. Esterase inhibition attribute of grapefruit juice leading to a new drug interaction. Drug Metab Dispos. 2007a;35(7):1023-31.

[10] Li P, Callery PS, Gan LS, Balani SK. Esterase inhibition by grapefruit juice flavonoids leading to a new drug interaction. Drug Metab Dispos. 2007b;35(7):1203-8.

[11] Nishimuta H, Tsujimoto M, Ogura K, Hiratsuka A, Ohtani H, Sawada Y. Inhibitory effects of various beverages on ritodrine sulfation by recombinant human sulfotransferase isoforms SULT1A1 and SULT1A3. Pharm Res. 2005;22(8):1406-10.

[12] Nishimuta H, Ohtani H, Tsujimoto M, Ogura K, Hiratsuka A, Sawada Y. Inhibitory effects of various beverages on human recombinant sulfotransferase isoforms SULT1A1 and SULT1A3. Biopharm Drug Dispos. 2007;28(9):491-500.

[13] Kim E, Sy-Cordero A, Graf TN, Brantley SJ, Paine MF, Oberlies NH. Isolation and identification of intestinal CYP3A inhibitors from cranberry (*Vaccinium macrocarpon*) using human intestinal microsomes. Planta Med. 2011;77(3):265-70.

[14] Roth M, Araya JJ, Timmermann BN, Hagenbuch B. Isolation of modulators of the liver-specific organic anion-transporting polypeptides (OATPs) 1B1 and 1B3 from *Rollinia emarginata* Schlecht (Annonaceae). J Pharmacol Exp Ther. 2011;339(2):624-32.

[15] Chen C, Cancalon P, Haun C, Gmitter, Jr., F. Characterization of furanocoumarin profile and inheritance toward selection of low furanocoumarin seedless grapefruit cultivars. JASHS. 2011;136(5):358-363.

[16] Brand W, Schutte ME, Williamson G, van Zanden JJ, Cnubben NH, Groten JP, *et al*. Flavonoid-mediated inhibition of intestinal ABC transporters may affect the oral bioavailability of drugs, food-borne toxic compounds and bioactive ingredients. Biomed Pharmacother. 2006;60(9):508-19.

[17] Heikkinen AT, Korjamo T, Mönkkönen J. Modelling of drug disposition kinetics in in vitro intestinal absorption cell models. Basic Clin Pharmacol Toxicol. 2010;106(3):180-8.

[18] Takanaga H, Ohnishi A, Matsuo H, Murakami H, Sata H, Kuroda K, *et al*. Pharmacokinetic analysis of felodipine-grapefruit juice interaction based on an irreversible enzyme inhibition model. Br J Clin Pharmacol. 2000;49(1):49-58.

[19] Fenneteau F, Poulin P, Nekka F. Physiologically based predictions of the impact of inhibition of intestinal and hepatic metabolism on human pharmacokinetics of CYP3A substrates. J Pharm Sci. 2010;99(1):486-514.

[20] Uesawa Y, Abe M, Fukuda E, Baba M, Okada Y, Mohri K. Construction of a model to estimate the CYP3A inhibitory effect of grapefruit juice. Pharmazie. 2011;66(7):525-528.

[21] Khan IA, Smillie T. Implementing a "quality by design" approach to assure the safety and integrity of botanical dietary supplements. J Nat Prod. 2012;75(9):1665-1673.

[22] FDA Guidance on Drug Interaction Studies. February 2012. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf.

[23] Chen ST, Dou J, Temple R, Agarwal R, Wu KM, Walker S. New therapies from old medicines. Nat Biotechnol. 2008;26(10):1077-1083.

[24] Dentali SJ. Successful botanical research requires botanical expertise. Clin Pharmacol Ther. 2010;87(2):149-151.

APPENDICES

APPENDIX A: PILOT STUDY EVALUATING POMEGRANATE JUICE AS AN INHIBITOR OF CYP3A ACTIVITY IN HEALTHY VOLUNTEERS

METHODS

Materials and chemicals. Pomegranate juice (brand D) was obtained from a local grocery store; brand D was selected based on results from preliminary studies [1] involving multiple brands of pomegranate juice (designated A-D), human intestinal microsomes, and midazolam 1'-hydroxylation as an index of CYP3A activity. Midazolam syrup was purchased from Roxane Laboratories, Inc. (Columbus, OH) and dispensed by the Department of Pharmacy's Investigational Drug Services at the University of North Carolina Hospitals. Methanol, water, acetic acid, ammonium hydroxide, and acetonitrile were of the highest grade and purchased from Fisher Scientific, Inc. (Fair Lawn, NJ). Midazolam, 1'-hydroxymidazolam, and alprazolam were purchased from Sigma-Aldrich (St. Louis, MO). Blank human plasma used to prepare calibration standards and quality controls was purchased from Biological Specialty Corporation (Colmar, PA).

Human Volunteer Study

Study protocol and subjects. The study protocol was reviewed and approved by the University of North Carolina Office of Human Research Ethics/Biomedical Institutional Review Board and Clinical Research Advisory Committee. Written informed consent was obtained prior to participation. Three healthy men and three non-pregnant women were enrolled. The men were between 35 and 58 years-old, and the women between 25 and 53 years-old. The participants were self-identified as white (2 men, 3 women) and African American (1 man). Prior to enrollment, each participant underwent a medical history, physical exam, liver function tests, basic metabolic panel, and complete blood count. Each woman also underwent a pregnancy test. None of the participants were taking drugs known to

modulate of CYP3A activity, except for 1 woman (white) who was taking oral contraceptives. Other concomitant medications included hydrochlorothiazide (1 white woman) and multivitamin (1 white woman). All participants were non-smokers.

Preparation of pomegranate juice (brand D). The selected pomegranate juice was fresh pressed and did not require reconstitution. The juice for each subject was dispensed from one 946 mL bottle of juice from the same lot.

Study design. Eligible subjects participated in a prospective, randomized, crossover, open-label study conducted at the UNC General Clinical Research Center (GCRC). The participants were instructed to abstain from all fruit juices one week prior to and during the study and from alcohol and caffeinated beverages the evening prior to each study day. Participants were admitted to the GCRC the evening prior to each of two study phases, which were separated by at least one week. Following an overnight fast, each subject was administered three 240-mL glasses of water or pomegranate juice, each separated by a 15-min interval. The subject ingested 5 mg midazolam syrup with the third glass. Blood (7 mL) was collected by venipuncture from an indwelling catheter at the following times: pre-dose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h post-dose. Vital signs (blood pressure, temperature, pulse, respiration rate, and oxygen saturation) were obtained at baseline and monitored periodically. Participants returned for an outpatient blood draw at 24 h post-dose. Blood was centrifuged within one hour of collection; plasma was drawn into cryovials and stored at -20°C pending analysis for midazolam and 1'-hydroxymidazolam.

Analysis of human plasma for midazolam and 1'-hydroxymidazolam. Concentrations of midazolam and 1'-hydroxymidazolam were determined using a modified, validated reverse-phase high-pressure liquid chromatography method with mass spectrometric detection [2, 3].

Pharmacokinetic analysis. The pharmacokinetics of midazolam and 1'-hydroxymidazolam were evaluated by non-compartmental methods using WinNonlin (v 5.0.1, Pharsight Corp., Mountain View, CA). The terminal elimination rate constant (λ_z) was estimated by log-linear regression of at least three data points in the terminal phase. The terminal half-life ($t_{½}$) was calculated as $0.693/\lambda_z$. The

maximum concentration (C_{max}) and time to reach C_{max} (t_{max}) were obtained directly from the concentration-time profile. The AUC from time zero to 12 hours (AUC_{0-12}) was determined using the trapezoidal rule with linear up/log down interpolation. The AUC from time zero to infinity ($AUC_{0-\infty}$) was determined by adding AUC_{last} and C_{last}/λ_z. The apparent oral clearance of midazolam (Cl/F) was calculated as the ratio of dose to $AUC_{0-\infty}$. Below limit of quantification (BLQ) concentrations were excluded from the data analysis.

Statistical analysis. The primary aim of the pilot clinical study was to calculate a point estimate and confidence interval of the difference (Δ) between water and pomegranate juice with respect to the disposition of midazolam. The study was exploratory in nature, and the sample size for the clinical study (n = 6) was deemed adequate. A two one-sided testing procedure, as recommended by the FDA Guidance for Industry regarding drug interaction studies, was utilized for testing [4]. Results are reported as 90% confidence intervals about the geometric mean ratios of the two observed pharmacokinetic measures, $AUC_{0-\infty}$ and C_{max}. For juice and water comparisons, midazolam + pomegranate juice was considered the test agent (numerator) and midazolam + water as the reference standard (denominator). The treatment groups were considered "bioequivalent" if the 90% confidence intervals around the estimated geometric mean ratios of the observed AUC and C_{max} were entirely within the 0.8 and 1.25 interval. Medians and ranges are reported for t_{max}. Geometric means and coefficients of variation are presented for C_{max}, AUC_{last}, $AUC_{0-\infty}$, Cl/F, and terminal $t_{½}$.

RESULTS

The effects of pomegranate juice D, a fresh pressed juice, were compared to water on the pharmacokinetics of midazolam in 6 healthy volunteers. Relative to water, the selected clinical test juice decreased the geometric mean C_{max} of midazolam by 32% and increased median t_{max} (by 1.5-fold). The geometric mean AUC of midazolam was decreased minimally (by ~10%). The terminal half-life was unchanged (Fig. A.1, Table A.1). The ratio of the point estimates of C_{max} and AUC did not meet bioequivalence acceptance limits.

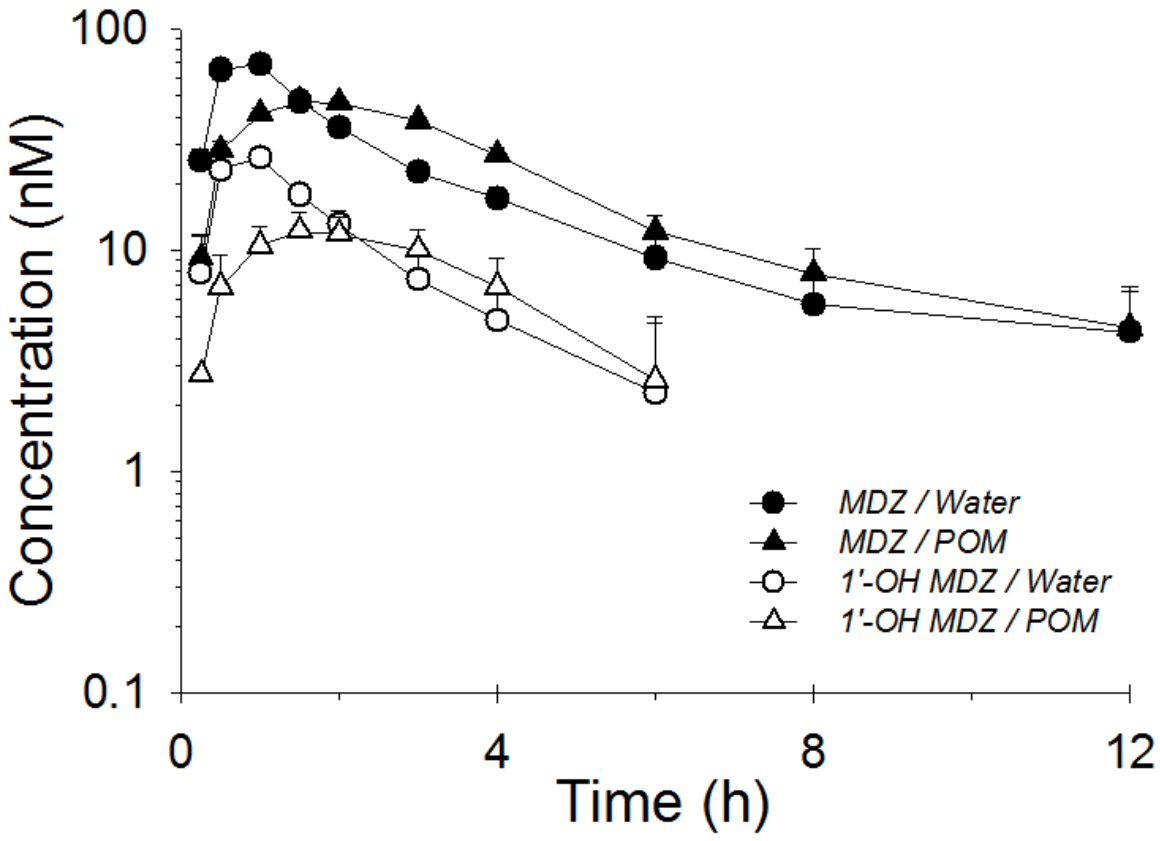


Fig. A.1 Geometric mean concentration-time profile of midazolam (MDZ) and 1'-hydroxymidazolam (1'-OH MDZ) for 6 healthy volunteers administered three 240-mL glasses of water or fresh pressed pomegranate juice brand D (POM) and a single dose of oral midazolam syrup (5 mg). Symbols and error bars denote geometric means and upper limits of the 90% confidence interval, respectively. The 6-h time point for MDZ during the water phase represents the geometric mean of 5 subjects.

Table A.1

Pharmacokinetics of midazolam and 1'-hydroxymidazolam in six healthy volunteers

Measure	Treatment		
	Water	POM	POM/Water Ratio
	Geometric Mean (CV %)		(90% CI)
Midazolam			
t_{max} (h) [median(range)]	1 (0.25-1)	1.5 (0.5-2)	
C_{max} (nM)	80.7 (42)	53.1 (18)	0.66 (-0.37-1.68)
AUC_{last} (nM ·h)	213 (36)	228 (19)	1.07 (0.09-2.05)
$AUC_{0-\infty}$ (nM ·h)	236 (32)	255 (20)	1.08 (0.10-2.06)
$t_{½}$ (h)	3.2 (29)	3.7 (32)	1.14 (0.57-1.70)
Cl/F (L/h)	65.1 (32)	60.1 (20)	0.92 (-0.06-1.90)
1'-Hydroxymidazolam			
t_{max} (h) [median(range)]	1 (0.5-1)	1.25 (0.5-2)	
C_{max} (nM)	30.0 (41)	13.8 (39)	0.46 (-1.01-1.93)
AUC_{last} (nM ·h)	59.7 (30)	51.6 (37)	0.86 (-0.41-2.14)
Metabolite/parent AUC ratio			
$(AUC_m/AUC_p)_{last}$	0.28 (8.5)	0.23 (12)	

POM, pomegranate juice; t_{max}, time to maximum concentration (C_{max}); AUC_{last}, area under the curve from time zero to last measurable concentration; $AUC_{0-\infty}$, area the curve from time zero to infinity; $t_{½}$, terminal half-life; Cl/F, apparent oral clearance

Due to lack of sensitivity to measure concentrations of 1'-hydroxymidazolam at later time points and subsequent insufficient capture of the terminal phase, $t_{½}$ and $AUC_{0-\infty}$ for 1'-hydroxymidazolam and $(AUC_m/AUC_p)_{0-\infty}$ were not calculable.

APPENDIX B: INHIBITION OF AN INTESTINAL ORGANIC ANION-TRANSPORTING POLYPEPTIDE BY ORANGE (*Citrus* × *sinensis* (L.) Osbeck), APPLE (*Malus* × *domestica* Borkh), AND POMEGRANATE (*Punica granatum* L.) JUICES

METHODS

Materials and chemicals. See Chapter 3.

Juice extraction. A variety of orange, apple, pomegranate, and cranberry juice brands were purchased from a local grocery store. For each juice, two 50-mL polypropylene tubes were filled with ~20 mL of juice. After adding ~20 mL of ethyl acetate to each tube, the tubes were shaken vigorously and centrifuged (5000 × *g* for 10 min at 25 °C). The upper organic layer was transferred to disposable glass tubes. This extraction process was repeated two more times for a total of three extractions for each juice. The ethyl acetate extract was dried under air and resuspended in methanol (500 μL) to yield a stock solution of 200X prior to testing.

Cell culture conditions. See Chapter 3

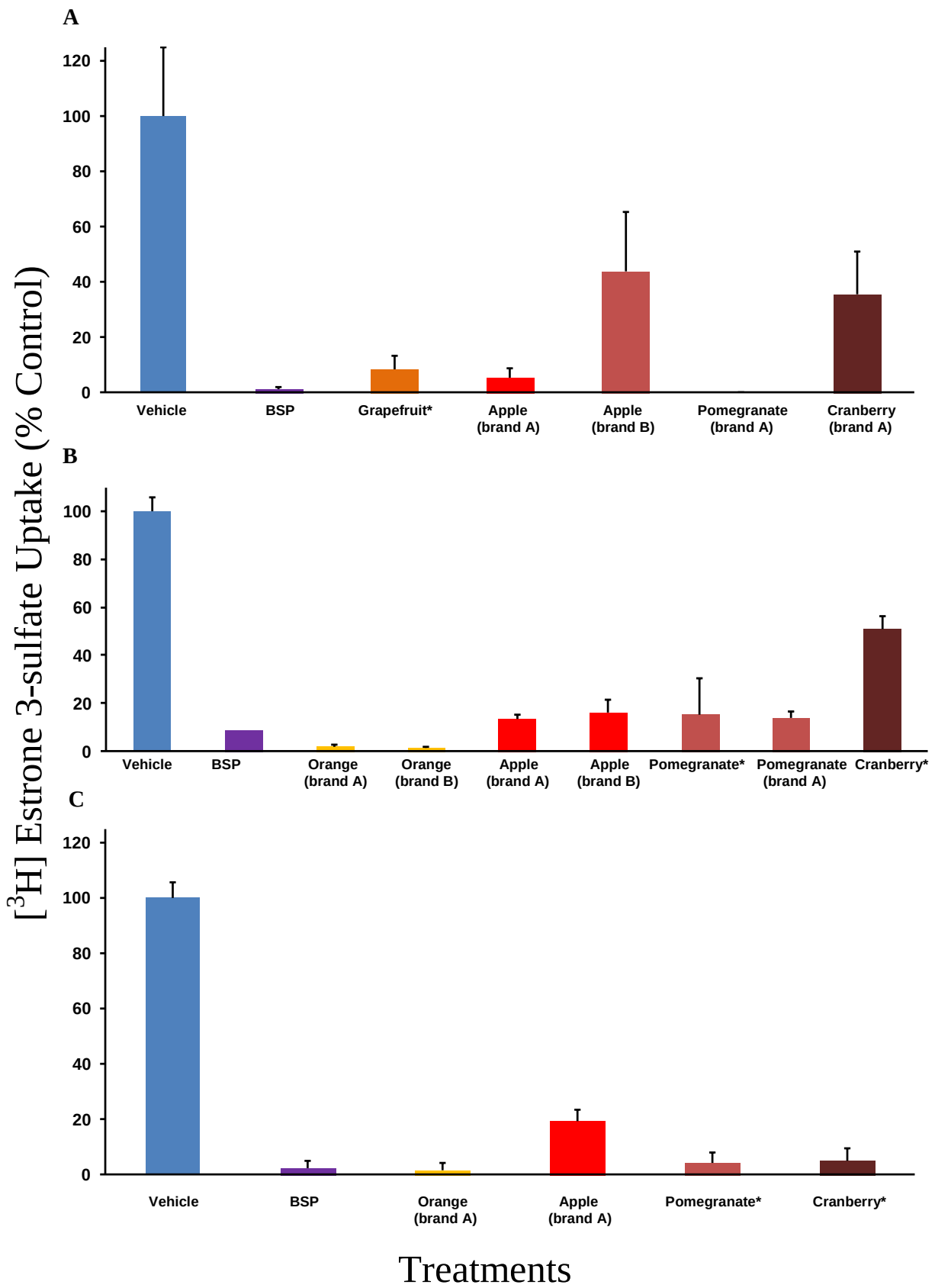
Screening of whole juice extracts for OATP2B1 inhibitory activity. See Chapter 3. Dosing solution (200 μL) consisted of radiolabeled plus unlabeled estrone 3-sulfate (total concentration, 0.5 μM) and juice extract (1X, 'single-strength'), the OATP inhibitor BSP (250 μM), or vehicle (2% methanol). Select whole juice extracts were tested at least three times on separate occasions to assess reproducibility.

Data analysis. Uptake values were corrected for protein content. OATP2B1-mediated net uptake was calculated by subtracting uptake values in parental cells from those in OATP2B1-expressing cells incubated under parallel conditions. The percent of control OATP activity was calculated by dividing values from experiments in the presence of inhibitor by values from experiments in the presence of vehicle and multiplying by 100.

Statistical analysis. Data are presented as means ± standard deviations of triplicate determinations.

RESULTS

Extracts of orange, apple, pomegranate, and cranberry juices inhibited OATP2B1-mediated estrone 3-sulfate uptake activity by up to 98% at 1X, relative to control (Fig. B.1). Orange juice brands A and B inhibited estrone 3-sulfate uptake by >98%. Apple juice brands A and B inhibited OATP2B1 activity by 56-86%. Pomegranate juice brand A inhibited estrone 3-sulfate activity by 86-100%. Cranberry juice brand A inhibited estrone 3-sulfate uptake activity by 65-86%.


A
120
100
80
60
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20
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Vehicle
BSP
Grapefruit*
Apple (brand A)
Apple (brand B)
Pomegranate (brand A)
Cranberry (brand A)
B
100
80
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Vehicle
BSP
Orange (brand A)
Orange (brand B)
Apple (brand A)
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Orange (brand A)
Apple (brand A)
Pomegranate*
Cranberry*
[3H] Estrone 3-sulfate Uptake (% Control)
Treatments

Fig. B.1 Effects of orange, apple, pomegranate, and cranberry juice extracts ('single-strength', 1X) on OATP2B1-mediated uptake of estrone 3-sulfate in stably transfected MDCKII cells on three separate occasions (A-C). Control activities for A, B, and C were 12.2 ± 3.0, 13.3 ± 0.8, and 9.5 ± 2.2 pmol/mg protein/2 min, respectively. * denotes juice used in a previous clinical study (pomegranate: Appendix A; cranberry: [3]). Bars and error bars denote means and SDs, respectively, of triplicate determinations. Bromosulfophthalein (BSP, 250 μM) and grapefruit juice extract (1X) were used as positive controls.

APPENDIX C: BIOACTIVITY-GUIDED FRACTIONATION OF ORANGE (*Citrus* × *sinensis* (L.) Osbeck), APPLE (*Malus* × *domestica* Borkh), AND POMEGRANATE (*Punica granatum* L.) JUICES TO IDENTIFY INHIBITORS OF ORGANIC ANION-TRANSPORTING POLYPEPTIDE 2B1

METHODS

Materials and chemicals. See Chapter 3.

Juice extraction. See Chapter 3.

Cell culture conditions. See Chapter 3.

Screening of juice fractions for OATP2B1 inhibitory activity. See Chapter 3. Dosing solution (200 μL) consisted of radiolabeled plus unlabeled estrone 3-sulfate (total concentration, 0.5 μM) and juice fraction (10, 50, or 100 g/mL), the OATP inhibitor BSP (250 μM), or vehicle (0.5-1% methanol).

Data analysis. Uptake values were corrected for protein content. OATP2B1-mediated net uptake was calculated by subtracting uptake values in parental cells from those in OATP2B1-expressing cells incubated under parallel conditions. The percent of control OATP activity was calculated by dividing values from experiments in the presence of inhibitor by values from experiments in the presence of vehicle and multiplying by 100.

Statistical analysis. Data are presented as means ± standard deviations of triplicate determinations.

RESULTS

The orange juice organic fraction contained the most active subfractions (A, G-J), which inhibited estrone 3-sulfate uptake by > 60% at 50 µg/mL (Fig. C.1). The butanol fraction inhibited estrone 3-sulfate uptake by ~80% at 50 µg/mL. The organic, aqueous, and butanol fractions of apple juice inhibited estrone 3-sulfate uptake by ≥ 80% at 50 µg/mL (Fig. C.2). The organic fraction contained two active subfractions (D, E), which inhibited estrone 3-sulfate uptake by > 60%. The organic fraction of pomegranate juice was the most potent, inhibiting estrone 3-sulfate uptake by ≥ 40% at 10 µg/mL (Fig. C.3). The butanol fraction inhibited estrone 3-sulfate uptake by ~40 and 75% at 50 and 100 µg/mL, respectively. Further fractionation of the organic and butanol fractions yielded two and four active fractions, respectively. Organic subfractions (A, E) inhibited estrone 3-sulfate uptake by > 60% at 50 µg/mL. Subfraction E inhibited by > 50% at 10 and 50 µg/mL. Butanol subfractions (D-G) inhibited estrone 3-sulfate uptake by ≥ 70 at 50 µg/mL. Subfractions E, F, and G inhibited by ≥ 60% at 10 and 50 µg/mL. In general, concentration-dependent OATP2B1 inhibition within each treatment for each juice was observed.

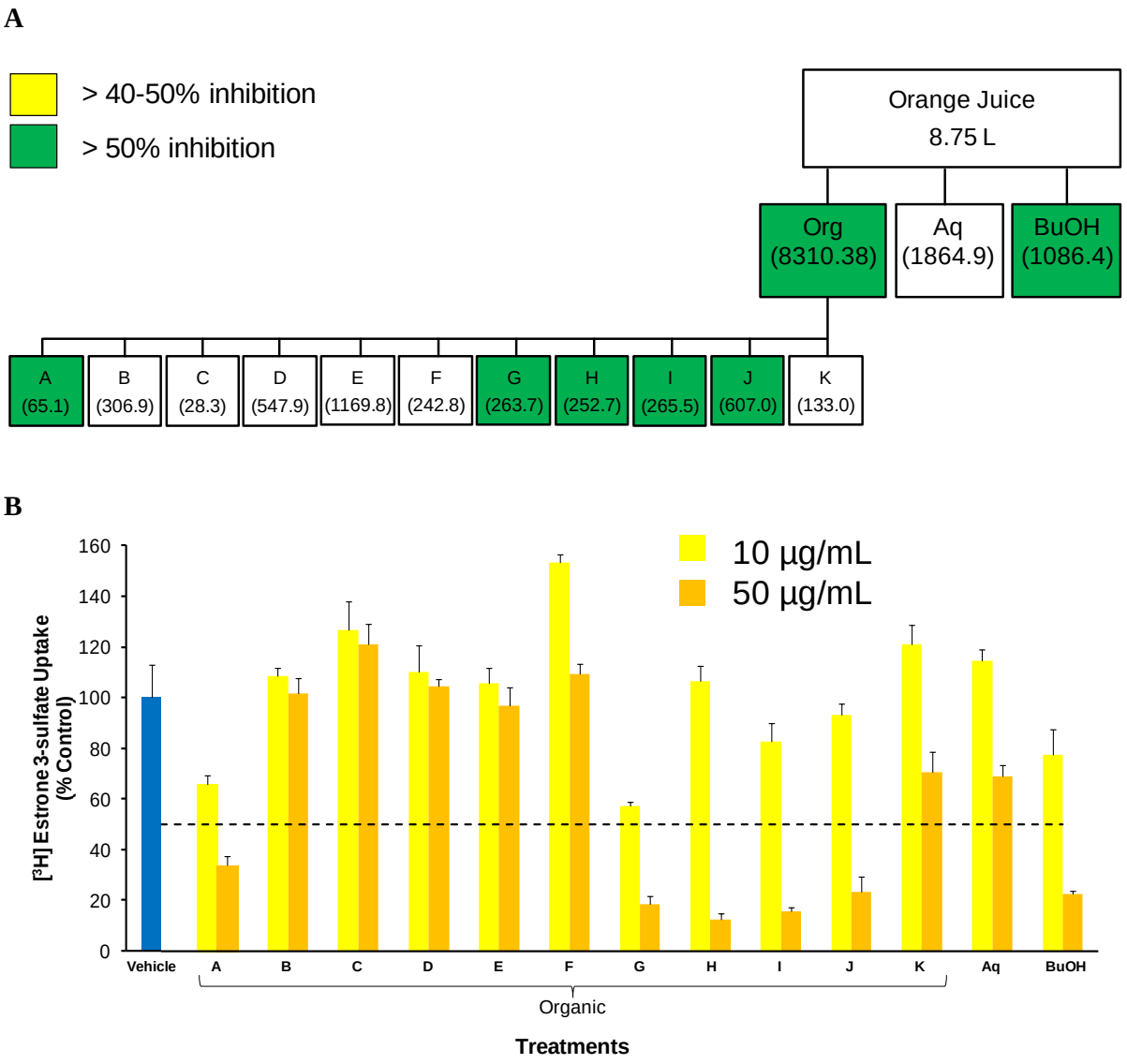


Fig. C.1 Separation scheme for bioactivity-guided fractionation of orange juice extracts (A) and effects of juice extracts and fractions on OATP2B1-mediated uptake of estrone 3-sulfate in stably transfected MDCKII cells (B). Numbers in parentheses are weights in mg. Aq, aqueous; BuOH, butanol; Org, organic (ethyl acetate). Bars and error bars denote means and SDs of triplicate incubations. Control activity was 11.3 ± 1.4 pmol/mg protein/2 min. Bromosulfophthalein (BSP, 250 µM) was used as a positive control and inhibited by 97%.

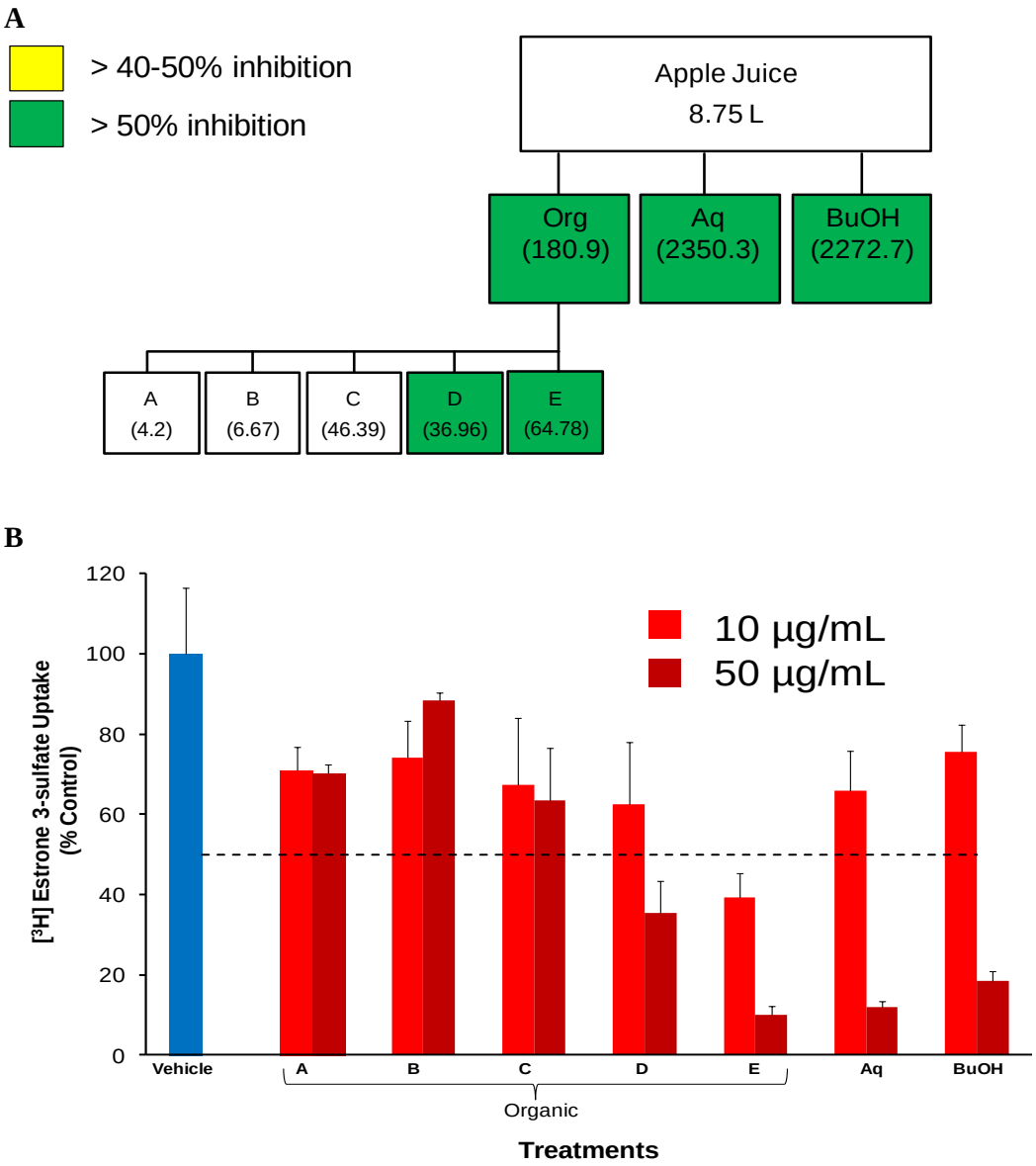
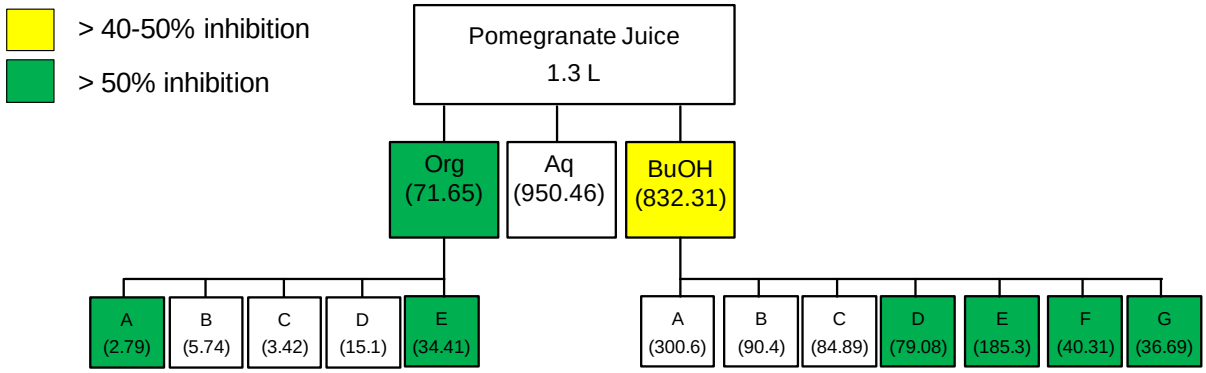


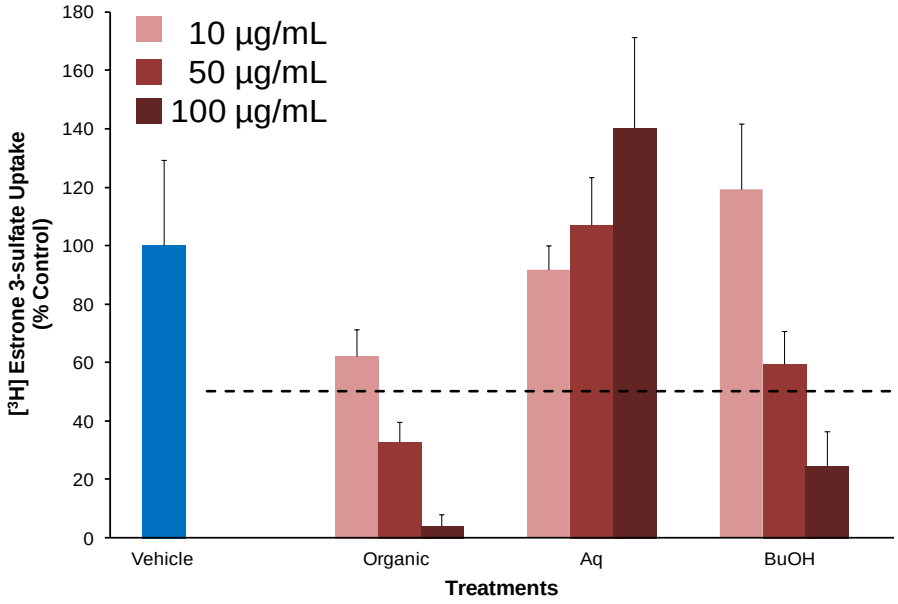
Fig. C.2 Separation scheme for bioactivity-guided fractionation of apple juice extracts (A) and effects of juice extracts and fractions on OATP2B1-mediated uptake of estrone 3-sulfate in stably transfected MDCKII cells (B). Numbers in parentheses are weights in mg. Aq, aqueous; BuOH, butanol; Org, organic (ethyl acetate). Bars and error bars denote means and SDs of triplicate incubations. Control activity was 100 ± 16 pmol/mg protein/2 min. Bromosulfophthalein (BSP, 250 µM) was used as a positive control and inhibited by 98%.

A



B

Round 1



Round 2

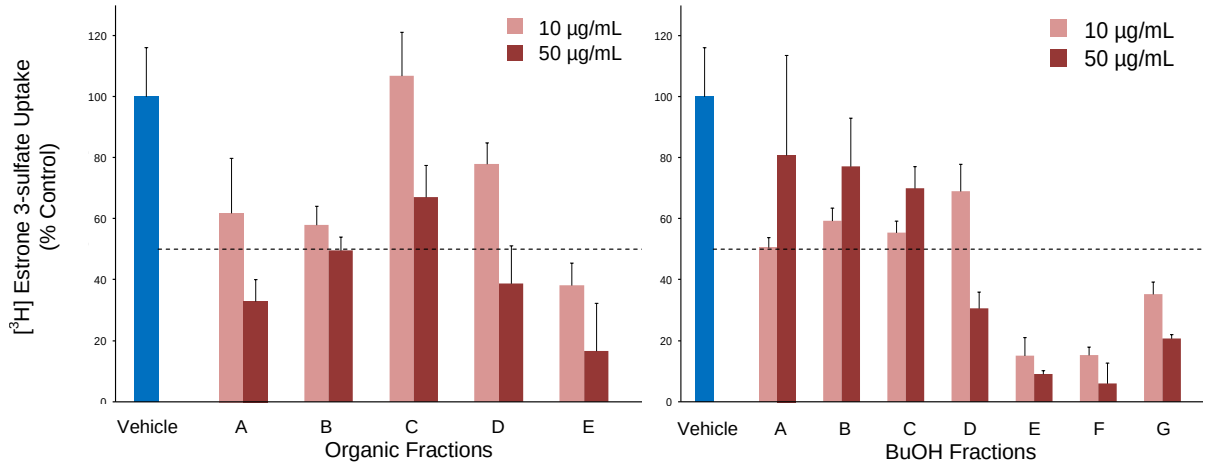


Fig. C.3 Separation scheme for bioactivity-guided fractionation of pomegranate juice extracts (A) and effects of juice extracts and fractions on OATP2B1-mediated uptake of estrone 3-sulfate in stably transfected MDCKII cells (B). Numbers in parentheses are weights in mg. Aq, aqueous; BuOH, butanol; Org, organic (ethyl acetate). Bars and error bars denote means and SDs of triplicate incubations. Control activities in Rounds 1 and 2 were 5 ± 0.9 pmol/mg protein/2 min and 15.4 ± 2.4 pmol/mg protein/2 min, respectively. Bromosulfophthalein (BSP, 250 μM) was used as a positive control and inhibited by 87 and 96%, respectively, in Rounds 1 and 2.

APPENDIX D: EVALUATION OF ATENOLOL AND CIPROFLOXACIN AS SUBSTRATES AND INHIBITORS OF ORGANIC ANION-TRANSPORTING POLYPEPTIDE 2B1

METHODS

Materials and chemicals. See Chapter 3. [2-^{14}C]Ciprofloxacin (15 mCi/mmol) and [ring-^{3}H]atenolol (5.1 Ci/mmol) were purchased from Moravek Radiochemicals (Brea, CA). Atenolol, ciprofloxacin, bromosulfophthalein (BSP), and D-glucose were purchased from Sigma-Aldrich (St. Louis, MO). Hanks' balanced salt solution (HBSS) with calcium and magnesium was purchased from Mediatech Inc. (Hendon, VA).

Cell culture conditions. See Chapter 3

Transport assays in stably transfected MDCKII cells. *Inhibition of estrone 3-sulfate by ciprofloxacin and atenolol*. Dosing solution (200 μL) consisted of radiolabeled plus unlabeled estrone 3-sulfate (total concentration, 0.5 μM) and ciprofloxacin or atenolol (5, 10, or 500 μM), the OATP inhibitor BSP (250 μM), or vehicle (0.04% methanol). Concentrations of atenolol and ciprofloxacin were chosen based on the only published information available, which were previous studies involving OATP1A2 and Oatp1a5 [5-7]. Cells were incubated for 2 min in pH 7.4 buffer at 37°C.

Determination of OATP2B1 involvement in ciprofloxacin and atenolol uptake. Dosing solution (200 μL) consisted of radiolabeled plus unlabeled ciprofloxacin (10 μM total) or atenolol (5 and 50 μM total). Cells were incubated over select time points up to 30 min in pH 6 and 7.4 buffer at 37°C.

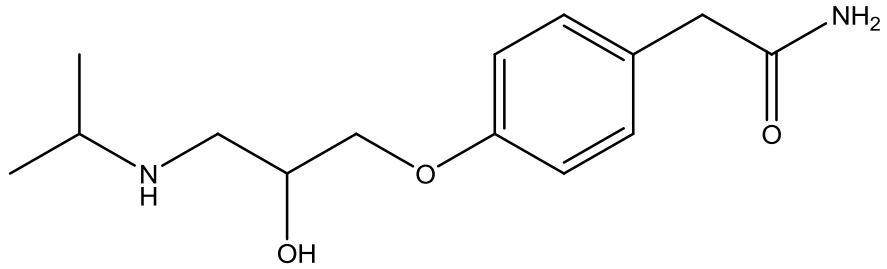
Data analysis. Uptake values were corrected for protein content. OATP2B1-mediated net uptake was calculated by subtracting uptake values in parental cells from those in OATP2B1-expressing cells incubated under parallel conditions. The percent of control OATP activity was calculated by dividing values from experiments in the presence of inhibitor by values from experiments in the presence of vehicle and multiplying by 100.

Statistical analysis. Data are presented as means ± standard deviations of triplicate determinations.

RESULTS

Ciprofloxacin and atenolol at 5, 10, and 500 μM did not inhibit OATP2B1-mediated estrone-3-sulfate uptake. Uptake studies with ciprofloxacin at pH 7.4 showed a 1.7-fold difference between OATP2B1-transfected and parental MDCKII cells at 1 min. Amounts of ciprofloxacin decreased progressively thereafter over 30 min. Uptake studies with atenolol showed amounts decreasing progressively in all conditions after 1-2 min. Initial uptake studies with atenolol 5 and 50 μM at pH 6.0 showed a 1.4- and 1.3-fold difference, respectively, between OATP2B1-transfected and parental MDCKII cells at 2 min. Uptake studies with atenolol 5 and 50 μM at pH 7.4 showed a 2.4- and 4.8-fold difference, respectively, between OATP2B1-transfected and parental MDCKII cells at 2 min. The (modified) repeat of one of the initial uptake studies with atenolol 5 and 50 μM (pH 7.4 only) showed no and a 1.4-fold difference, respectively, between OATP2B1-transfected and parental MDCKII cells at 1 min.

A



B

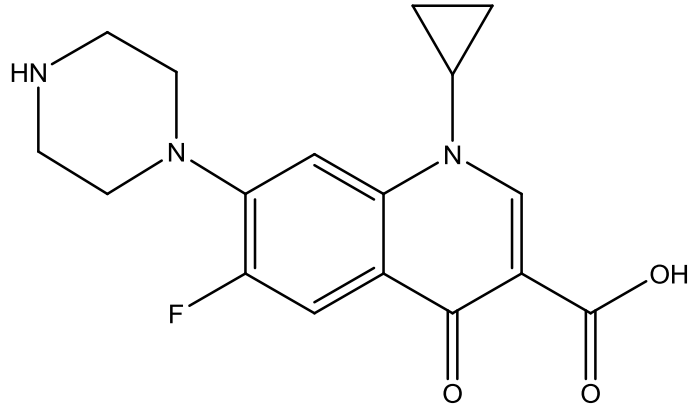


Fig. D.1 Structures of atenolol (A) and ciprofloxacin (B)

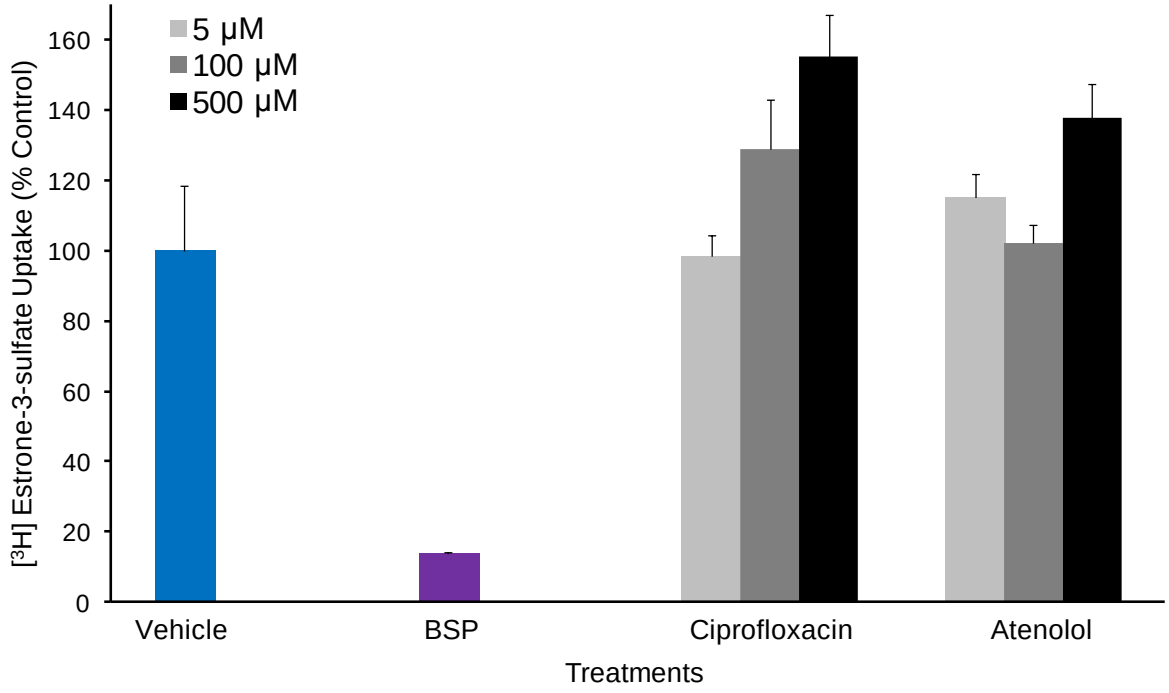


Fig. D.2 Inhibition of estrone 3-sulfate uptake by ciprofloxacin and atenolol in stably transfected MDCK II cells. Control activity was 17.4 ± 2.9 pmol/mg protein/2 min. Bars and error bars denote means and SDs, respectively, of triplicate determinations. Bromosulfophthalein (BSP, 250 µM) was used as a positive control.

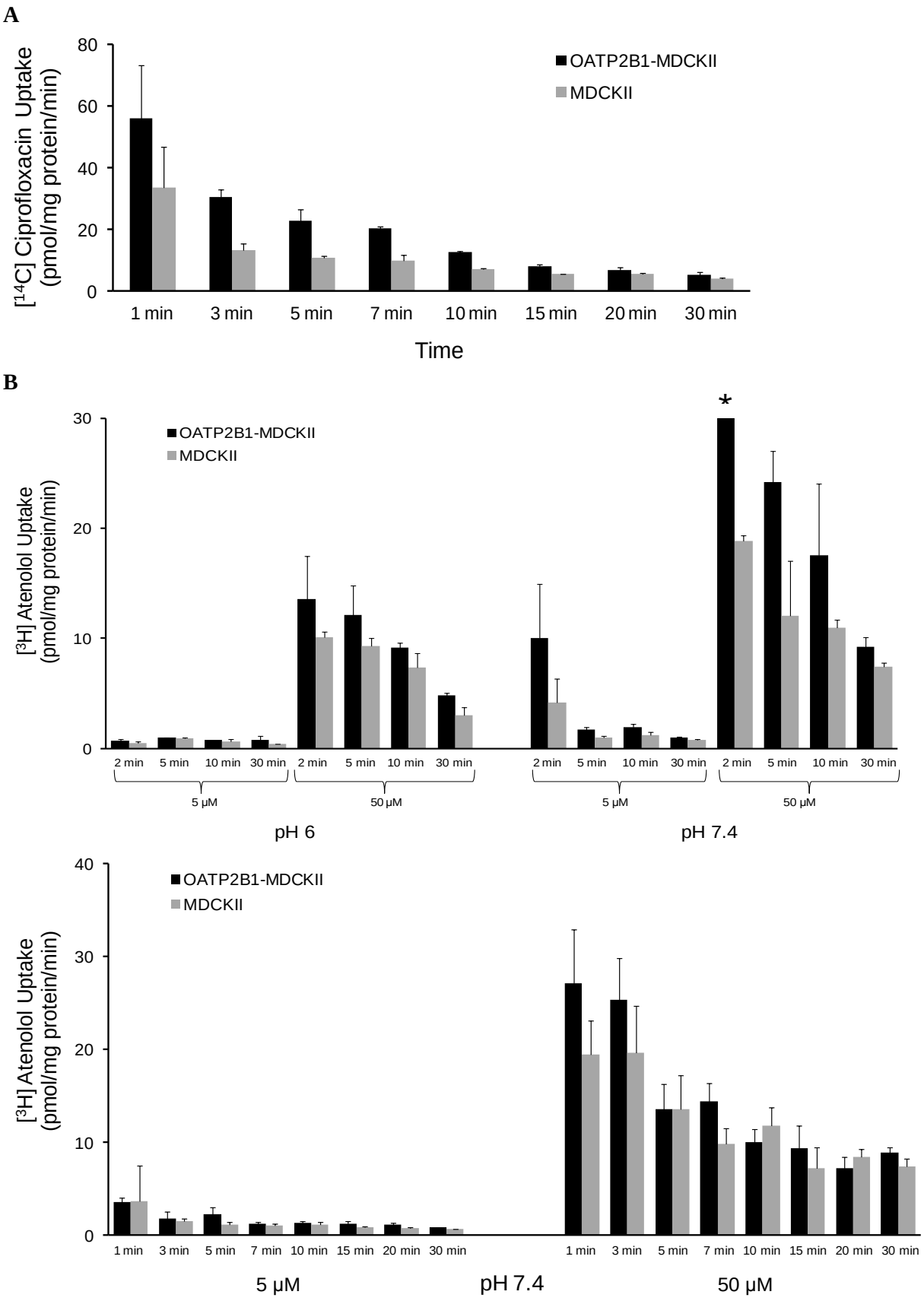

A
[14C] Ciprofloxacin Uptake (pmol/mg protein/min)
OATP2B1-MDCKII
MDCKII
1 min
3 min
5 min
7 min
10 min
15 min
20 min
30 min
Time
B
[3H] Atenolol Uptake (pmol/mg protein/min)
OATP2B1-MDCKII
MDCKII
*
2 min
5 min
10 min
30 min
5 µM
50 µM
pH 6
pH 7.4
[3H] Atenolol Uptake (pmol/mg protein/min)
OATP2B1-MDCKII
MDCKII
1 min
3 min
5 min
7 min
10 min
15 min
20 min
30 min
5 µM
pH 7.4
50 µM

Fig. D.3 Uptake of ciprofloxacin (A) and atenolol (B) in stably transfected and parental MDCKII cells. Bars and error bars denote means and SDs, respectively, of triplicate determinations, except for 10 min time point for atenolol 5 μM at pH 6 (n=2). * Uptake = 90.9 ± 21.2 pmol/mg protein/min.

APPENDIX E: INTERDAY VARIABILITY OF ESTRONE 3-SULFATE TRANSPORT IN TRANSFECTED MDCKII CELLS EXPRESSING ORGANIC ANION-TRANSPORTING POLYPEPTIDE 2B1

METHODS

Materials and chemicals. See Chapter 3.

Cell culture conditions. See Chapter 3.

Uptake assay in stably transfected MDCKII cells. See Chapter 3. Cells were incubated for 2 min in pH 7.4 buffer at 37°C.

Data analysis. Uptake values were corrected for protein content. OATP2B1-mediated net uptake was calculated by subtracting uptake values in parental cells from those in OATP2B1-expressing cells incubated under parallel conditions. The percent of control OATP activity was calculated by dividing values from experiments in the presence of inhibitor by values from experiments in the presence of vehicle and multiplying by 100.

Statistical analysis. Data are presented as means ± standard deviations of triplicate determinations.

Table E.1

Interday variability of OATP2B1-mediated estrone 3-sulfate uptake

OATP2B1-MDCKII Cells				
Date	**Methanol (%)**	Estrone 3-sulfate Uptake (pmol/mg protein/2min)	**BSP**	Estrone 3-sulfate Uptake (pmol/mg protein/2min)
5/5/2011	2	12.2 ± 3.0	250	0.13 ± 0.1
5/8/2011	1.7	13.3 ± 0.8		1.12*
5/28/2011	0.5	9.5 ± 2.2		0.2 ± 0.2
5/28/2011	1.7	10.9 ± 0.8		0 ± 0.2
7/1/2011	1	3.08 ± 0.9		0.4 ± 0.1
8/8/2011	0.5	15.1 ± 2.4		0.6 ± 1.0
8/11/2011	0.5	98.8 ± 16		2.5 ± 0.8
8/13/2011	0.5	10.7 ± 1.4		0.3 ± 0.1

Values are means ± SDs.

* Fruit juice testing in Appendices B and C.

† n=2

Bromosulfophthalein (BSP, 250 μM) was a positive control.

REFERENCES

[1] Yan Z, Oberlies NH, Paine MF. Extent of human enteric CYP3A inhibition in vitro by pomegranate juice varies with brand. AAPS PharmSci. 2007;9(S2). Available from: http://www.aapspharmsci.org/.

[2] Kanazawa H, Okada A, Igarashi E, Higaki M, Miyabe T, Sano T, Nishimura R. Determination of midazolam and its metabolite as a probe for cytochrome P450 3A4 phenotype by liquid chromatography mass spectrometry. J Chromatogr A 2004;1031:213-8.

[3] Ngo N, Yan Z, Graf TN, Carrizosa DR, Kashuba AD, Dees EC, Oberlies NH, Paine MF. Identification of a cranberry juice product that inhibits enteric CYP3A-mediated first-pass metabolism in humans. Drug Metab Dispos 2009;37:514-22.

[4] Huang SM, Temple R, Throckmorton DC, Lesko LJ. Drug interaction studies: study design, data analysis, and implications for dosing and labeling. Clin Pharmacol Ther 2007;81:298-304.

[5] Maeda T, Takahashi K, Ohtsu N, Oguma T, Ohnishi T, Atsumi R, Tamai I. Identification of influx transporter for the quinolone antibacterial agent levofloxacin. Mol Pharm. 2007;4:85-94.

[6] Kato Y, Miyazaki T, Kano T, Sugiura T, Kubo Y, Tsuji A. Involvement of influx and efflux transport systems in gastrointestinal absorption of celiprolol. J Pharm Sci. 2009;98:2529-39.

[7] Arakawa H, Shirasaka Y, Haga M, Nakanishi T, Tamai I. Active intestinal absorption of fluoroquinolone antibacterial agent ciprofloxacin by organic anion transporting polypeptide, Oatp1a5. Biopharm Drug Dispos. 2012;33:332-41.