1521-0081/73/2/847–859$35.00
PHARMACOLOGICAL REVIEWS
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https://doi.org/10.1124/pharmrev.120.000106
Pharmacol Rev 73:847–859, April 2021

ASSOCIATE EDITOR: HYUNYOUNG JEONG

Modeling Pharmacokinetic Natural Product–Drug Interactions for Decision-Making: A NaPDI Center Recommended Approach

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This work was supported by National Institutes of Health National Center for Complimentary and Integrative Health and Office of Dietary Supplements [Grant U54-AT008909] and in part by National Institute on Drug Abuse [Grant P01-DA032507]. E.J.C. was formerly a postdoctoral research associate with the Center of Excellence for Natural Product Drug Interaction Research (NaPDI Center) and is now a paid medical writer for the NaPDI Center.

The authors have no financial conflicts of interest to disclose.

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https://doi.org/10.1124/pharmrev.120.000106.

***Abstract*——The popularity of botanical and other purported medicinal natural products (NPs) continues to grow, especially among patients with chronic illnesses and patients managed on complex prescription drug regimens. With few exceptions, the risk of a given NP to precipitate a clinically significant pharmacokinetic NP-drug interaction (NPDI) remains understudied or unknown. Application of static or dynamic mathematical models to predict and/or simulate NPDIs can provide critical information about the potential clinical significance of these complex interactions. However, methods used to conduct such predictions or simulations are highly variable. Additionally, published reports using mathematical models to interrogate NPDIs are not always sufficiently detailed to ensure reproducibility. Consequently, guidelines are needed to inform the conduct and reporting of these modeling efforts. This recommended approach from the Center of Excellence for Natural Product Drug Interaction Research describes a systematic method for using mathematical models to interpret the interaction risk of NPs as precipitants of potential clinically significant pharmacokinetic NPDIs. A framework for developing and applying pharmacokinetic NPDI models is presented with the aim of promoting accuracy, reproducibility, and generalizability in the literature.**

***Significance Statement*——Many natural products (NPs) contain phytoconstituents that can increase or decrease systemic or tissue exposure to, and potentially the efficacy of, a pharmaceutical drug; however, no regulatory agency guidelines exist to assist in predicting the risk of these complex interactions. This recommended approach from a multi-institutional consortium designated by National Institutes of Health as the Center of Excellence for Natural Product Drug Interaction Research provides a framework for modeling pharmacokinetic NP-drug interactions.**

I. Introduction: Application of Static and Dynamic Models to Natural Products

Static and dynamic [i.e., physiologically-based pharmacokinetic (PBPK)] models are mainstay tools during drug development. For applications such as estimating dissolution and bioavailability, triaging early-phase new chemical entities (NCEs) with suboptimal pharmacokinetic characteristics (e.g., high clearance or low oral bioavailability), or predicting drug-drug interactions (DDIs), PBPK models can be used to design and occasionally replace clinical studies (Sager et al., 2015). Botanical dietary supplements and other purported medicinal natural products (NPs) often contain phytoconstituents that can precipitate clinically significant pharmacokinetic and potential pharmacodynamic NP-drug interactions (NPDIs) with conventional medications (both approved prescription and nonprescription) (Grimstein and Huang, 2018; Johnson et al., 2018; Paine et al., 2018). NPs can also contain misidentified plants or toxic chemical constituents introduced through suboptimal harvesting, production, and/or manufacturing practices (van Breemen et al., 2008). Induction or inhibition of cytochrome P450 (CYP) 3A by St. John's wort or grapefruit juice, respectively, are textbook examples of NPDIs that can increase or decrease the systemic exposure to CYP3A object drugs (Bailey et al., 1998; Henderson et al., 2002).

As with DDIs, NPDIs can perturb object drug systemic exposure to subtherapeutic or supratherapeutic concentrations, which in turn can lead to altered therapeutic response to the drug. However, mathematical modeling of NPDIs has not kept pace with that of DDIs. Unlike DDIs, to date, NPDI prediction is not driven by guidance documents from regulatory agencies, including the US Food and Drug Administration (FDA), European Medicines Agency, and the Pharmaceuticals and Medical Devices Agency. Silence on this challenging topic may have arisen from the intricacies of NPDI modeling and simulation, which require special attention to the phytochemical complexity of NPs, inconsistencies in formulations, differences in botanical taxonomy and nomenclature, and the paucity of human pharmacokinetic data for most commercially available NPs.

Despite the absence of guidance documents, static and PBPK models for estimating changes in object-drug systemic exposure have been developed (Zhou et al., 2005; Brantley et al., 2013; Ainslie et al., 2014; Brantley et al., 2014b; Gufford et al., 2015a; Tian et al., 2018; Adiwidjaja et al., 2019, 2020b). That NPDI models continue to be developed in the absence of regulatory guidance underscores the timeliness and importance of NPDI modeling and simulation and the need for resources and guidelines to support this research effort.

Compared with DDIs, NPDIs remain uniquely difficult to predict because of several key factors that preclude accurate in vitro-to-in vivo extrapolation: 1) the inherently complex and variable composition of phytoconstituents among marketed products of presumably the same NP, 2) identification of all possible constituents that contribute to NPDIs, 3) the often relatively sparse human pharmacokinetic information about precipitant ("perpetrator")

ABBREVIATIONS: AUC, area under the concentration-versus-time curve; DDI, drug-drug interaction; F_a, fraction of oral dose absorbed into the intestinal wall; FDA, US Food and Drug Administration; f_u, fraction unbound; HLM, human liver microsome; K_I, inhibitor concentration at half maximum inactivation rate; K_i, reversible inhibition constant; $K_{i,u}$, unbound reversible inhibition constant; k_{inact}, maximum inactivation rate constant; NaPDI Center, Center of Excellence for Natural Product Drug Interaction Research; NCE, new chemical entity; NP, natural product; NPDI, NP-drug interaction; PBPK, physiologically-based pharmacokinetic; UGT, UDP-glucuronosyltransferase.

NP constituents, and 3) potentially complex and varying interactions between the precipitants (e.g., synergy between constituents, inhibition by one constituent, and induction by another) due to the variable composition of precipitants in the same NP (Grimstein and Huang, 2018; Paine et al., 2018; Sorkin et al., 2020). The limited plasma exposure data for most commercially available NPs as well as the general absence of physicochemical data for their major phytoconstituents are perhaps the greatest impediments to developing robust PBPK models in this field. Indeed, the FDA recognizes these deficiencies as "technical challenges in determining standard pharmacokinetic measurements (https://www.fda.gov/media/93113/download)." This recommended approach lays a framework for selection of robust in vitro data, appropriate model parameterization and verification, and clear communication of model characteristics in the literature with the aim of promoting accuracy, reproducibility, and generalizability of pharmacokinetic NPDI models.

Recognizing that NPDIs are a pressing but understudied public health risk, the National Center for Complementary and Integrative Health established the Center of Excellence for Natural Product Drug Interaction Research (NaPDI Center), which is tasked with developing recommended approaches to guide researchers on the conduct of rigorous NPDI studies (Paine et al., 2018). The NaPDI Center has released recommended approaches for selecting and prioritizing NPs as potential precipitants of NPDIs and for sourcing and characterizing NPs for research studies (Johnson et al., 2018; Kellogg et al., 2019). This recommended approach summarizes existing challenges and potential solutions related to mathematical modeling of pharmacokinetic NPDIs with the goal of facilitating more rapid and systematic identification of clinically significant NPDIs.

II. Generating and Selecting Data for Static and Physiologically Based Pharmacokinetic Models

A. Identification of Precipitant Phytoconstituents

For many commercial NPs, precipitant phytoconstituent(s) (i.e., inducers and inhibitors of drug metabolizing enzymes and transporters) may not have been identified. These situations merit judicious sourcing and characterization of the crude NP followed by identification and quantification of precipitant constituents. One of the NaPDI Center's recommended approaches details pivotal considerations for sourcing and characterizing NPs for both in vitro and in vivo studies involving an NP (Kellogg et al., 2019). These considerations mirror those put forth by the FDA for ensuring therapeutic consistency and quality control during botanical drug development (https://www.fda.gov/media/93113/download) and by National Center for Complementary and Integrative Health for promoting consistency in grant applications and research reporting (https://nccih.nih.gov/research/policies/naturalproduct.htm#requestedpi).

Identifying phytoconstituents as precipitants of pharmacokinetic NPDIs is a complex and variable process, which typically includes a screening and/or experimental approach involving human-derived in vitro systems expressing relevant drug metabolizing enzymes and/or transporters. Experimental approaches include iterative fractionation and screening of crude extracts, during which an NP is partitioned into aqueous and organic phases and separated chromatographically into discrete pools of phytochemicals. These fractions are subsequently tested for bioactivity (induction or inhibition) across a predefined array of concentrations against a panel of drug metabolizing enzymes and transporters. Such biochemometric analysis or bioactivity-directed fractionation allows the bioactive fraction(s) to be refined and rescreened iteratively, progressively isolating fractions containing relatively purified mixtures of bioactive constituents or highly purified individual constituents (Kim et al., 2011; Kellogg et al., 2016; Rivera-Chávez et al., 2017a,b, 2019a,b; Amrine et al., 2018; Britton et al., 2018; Caesar et al., 2018; Tian et al., 2018; El-Elimat et al., 2019; Paguigan et al., 2019).

If the NP constituents are known and corresponding chemical structures are available, structure-activity comparisons may be used to anticipate the likelihood of NPDIs based solely on the presence of certain functional groups in individual constituent structures (Johnson et al., 2018) (Table 1). For example, methylenedioxyphenyl groups are well known structural alerts for potential time-dependent inhibition of the cytochrome P450 enzymes that involve stable heme coordination, whereas catechol groups or α,β-unsaturated aldehydes and ketones are structural alerts for time-dependent inhibition of cytochrome P450 enzymes that produce reactive intermediates and covalent protein adduction (Johnson et al., 2018).

B. Obtaining Existing Data to Populate Static and Physiologically-Based Pharmacokinetic Models with Requisite Parameters

1. Collecting Physicochemical Data. Several open-source and/or commercial screening libraries exist specifically for the purpose of collating physicochemical characteristics of NPs (Gao et al., 2008; Valli et al., 2013; Mirza et al., 2015; Xie et al., 2015; Chen et al., 2018; Pilón-Jiménez et al., 2019). These databases are designed primarily to facilitate in silico identification of NCEs and to obtain experimentally determined characteristics, including structure, pK_a, logarithm of octanol:water partition ratio, stereochemistry, and possible mechanisms of action. Additionally, the CHEMFATE data base curates available physicochemical data for many chemical entities (https://cfpub.epa.gov/si/si_public_record_Report.cfm?Lab=&dirEntryID=2897).

For constituents whose physicochemical characteristics have not been determined experimentally, structure-based prediction of chemical properties can be made provided that the molecular structure is known. Structure-based

TABLE 1
Structural alerts for constituents in select natural products
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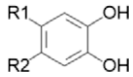
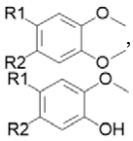
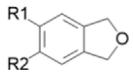
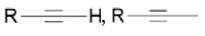
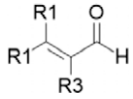
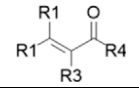
Constituent(s)/Natural Product	Structural Alert	Alert Substructure
Flavonoids, phenylpropanoids/*Echinacea* glycyrrhizin, glycyrrhizinic acid/licorice	Catechols	
Isoquinoline alkaloids/goldenseal terpenoids/cinnamon curcuminoids/turmeric	Masked catechol	
Isoquinoline alkaloids/goldenseal shizandrins/*Schisandra spp.* Gomisins/ *Schisandra spp.*	Methylenedioxyphenyl	
Cycloartenol/black cohosh	Subterminal olefin	
Polyacetylenes/*Echinacea*	Terminal and subterminal acetylenes	
Terpenoids/cinnamon diallyl disulfides and trisulfides/garlic	Terminal olefin	
Cinnamaldehyde/cinnamon	α,β-Unsaturated aldehyde	
Curcuminoids/turmeric	α,β-Unsaturated ketone	

prediction of phase partitioning has shown excellent coefficients of determination with direct measurement (r^2 = 0.51–0.91) (Eros et al., 2002; An et al., 2014; National Research Council, 2014), although performance is less accurate for phosphorus- and halogen-containing chemical entities (An et al., 2014). Similarly, pK_a can be predicted using a variety of computational tools (Voutchkova et al., 2012). The intestinal effective permeability and absorption rate constant (k_a) can be predicted from basic molecular attributes (polar surface area, phase partitioning, and hydrogen-bond donors), showing relatively high predictive performance with experimental F_a (fraction of the oral dose absorbed into the intestinal wall) values ($r^2 > 0.70$) (Winiwarter et al., 1998; Linnankoski et al., 2006). When an NP is formulated as a capsule or tablet, solubility and dissolution may be limiting factors for absorption. Alternatively, a conservative estimate of 100% F_a may be used to predict the highest degree of exposure to precipitant constituents.

In contrast to physicochemical factors, computational prediction of factors influencing distribution (e.g., plasma protein and tissue binding) remains less developed (Poulin, 2015a). Previous studies that estimated the extent of plasma protein binding using sigmoidal functions of logarithm of octanol:water partition ratio showed high predictive performance compared with direct measurement (r^2 = 0.79), whereas others have proposed simulating unbound drug concentrations in tissue compartments (Yamazaki and Kanaoka, 2004; Poulin, 2015b). Experimental methods for measuring the extent of plasma protein binding or fraction unbound (f_u) rely on long-established techniques for separating bound and unbound drug (Rowland, 1980). Until further research validates novel methods for simulating protein binding behavior of NP constituents, determining f_u experimentally is recommended based on data generated by the NaPDI Center (Nguyen et al., 2019). In brief, f_u for multiple NP constituents (n = 14–17) in human liver microsomes (HLMs) and plasma was generated in silico using two modeling and simulation platforms (www.certara.com, v17; Simcyp and www.simulations-plus.com/software/gastroplus, v9.6; GastroPlus) and compared with experimentally determined values. Experimental f_u was recovered via equilibrium dialysis using a 96-well device as described (Zamek-Gliszczynski et al., 2011). In silico–generated values ranged from 0.48 to 1.00 and from 0.01 to 0.75 in HLMs and plasma, respectively. Average (±S.D. of at least three determinations) experimental f_u ranged from 0.052 ± 0.008 to 1.21 ± 0.09 for HLMs and from 0.013 ± 0.003 to 0.95 ± 0.20 for plasma. The ratio of in silico–generated f_u values to experimental f_u values was assessed for low, moderate, and high binding constituents (Fig. 1). Experimental f_u for plasma proteins was generally lower than that for HLMs, which was consistent with values generated in silico. Both modeling and simulation platforms consistently predicted f_u values for low binding constituents to within 30% of experimental values, suggesting that in silico–generated values are

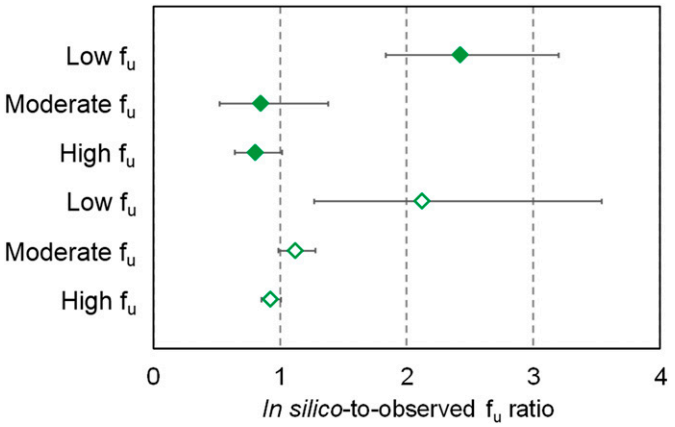


Fig. 1. Variability in the geometric mean of in-silico-to-observed f_u ratios for high binding (low f_u, denoted by experimental $f_u \leq 20\%$), moderate binding (moderate f_u, denoted by $20\% <$ experimental $f_u < 80\%$), and low binding (high f_u, denoted by experimental $f_u \geq 80\%$) natural product constituents in human liver microsomes and plasma. Error bars denote 90% confidence intervals. Closed diamonds denote values generated by GastroPlus, whereas open diamonds denote values generated by Simcyp. Natural product constituents evaluated are 4-methylumbelliferone, 7-hydroxymitragynine, berberine, bergamottin, hydrastine, hydrastinine, isosilybin A, isosilybin B, isosilychristin, mitraciliatine, mitragynine, paynantheine, silybin A, silybin B, silychristin, silydianin, and speciogynine.

reasonable estimates for low binding constituents. However, predictive performance diminished for moderate and high binding constituents. Continued comparisons of in silico–generated and experimental f_u values for additional NP constituents will form a database that can be used to develop predictive models of f_u as described for pharmaceutical drugs (Obach, 1999; Lombardo et al., 2018).

2. Generating Requisite Model Parameters from In Vitro Experiments. In general, based on the morphologic, transcriptomic, and proteomic differences among animal, immortalized human, and primary human tissues, the latter are the preferred experimental systems for characterizing xenobiotic metabolism and transport (Baillie and Rettie, 2011; Kauffman et al., 2013; Sawant-Basak et al., 2018). For these reasons, FDA guidance documents and the International Transporter Consortium recommend conducting in vitro DDI studies using human-derived systems or systems modified to express human drug-metabolizing enzymes and/or transporters (Brouwer et al., 2013; Chu et al., 2018; Evers et al., 2018; Zamek-Gliszczynski et al., 2018; FDA, 2020). These systems include recombinant enzymes, human subcellular tissue fractions (e.g., microsomes, cytosol), cell lines expressing human transporters, and intact human cell systems (i.e., hepatocytes, enterocytes). Recommended panels of enzymes and transporters against which potential NP precipitant constituents should be screened have been proposed in a previous NaPDI Center recommended approach (Johnson et al., 2018) (Table 2). In addition to the systems proposed in the earlier recommended approach, kidney- and intestine-derived microsomes and cell lines should be considered because of the well known interorgan differences in enzyme and transporter expression and function (Loretz et al., 2020).

TABLE 2
Recommended enzymes, transporters, and experimental systems for screening natural products for inhibition and/or induction

Adapted with permission from the American Society for Pharmacology and Experimental Therapeutics from Johnson et al. (2018).

Cytochrome P450 enzymes		
Essential: CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A		
Experimental System	Inhibition	Induction
Recombinant enzymes	As needed	NA
Human liver microsomes	[a]	NA
Human hepatocytes	As needed	[a]
Human intestinal microsomes	As needed	NA
Human intestinal cells	As needed	As needed[b]
Human kidney microsomes	As needed	NA
Human kidney cells	As needed	As needed[b]
Other cell lines	As needed	NA
UGTs		
Essential: UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B10, UGT2B15		
Experimental System	Inhibition	Induction
Recombinant enzymes	As needed	NA
Human liver microsomes	[a]	NA
Human hepatocytes	[a]	[a]
Human intestinal microsomes	As needed	NA
Human intestinal cells	As needed	As needed[b]
Human kidney microsomes	As needed	NA
Human kidney cells	As needed	As needed[b]
Other cell lines	As needed	NA
Other Enzymes that May Be Considered		
hCE1, hCE2, SULT1A1, SULT1A3, SULT1B1, SULT1E1, SULT2A1		
Experimental System	Inhibition	Induction
Recombinant enzymes	[a]	NA
Human liver microsomes	[a]	NA
Human hepatocytes	As needed	[a]
Human intestinal microsomes	As needed	NA
Human intestinal cells	As needed[b]	As needed[b]
Human kidney microsomes	As needed[b]	NA
Human kidney cells	As needed[b]	As needed[b]
Transporters		
Essential: BCRP, BSEP, MATE1, MATE2-K, MRP2, MRP3, NTCP, OATP1B1, OATP1B3, OATP2B1, OAT, OCT, P-gp		
Experimental System	Inhibition	Induction
Transfected cell lines (single, double)	[a]	NA
Human intestinal cells	As needed[b]	As needed[b]
Human kidney cells	As needed[b]	As needed[b]
Human hepatocytes	[a]	[a]
Membrane vesicles	[a]	NA

BCRP, breast cancer resistance protein; BSEP, bile salt export pump; hCE, human carboxylesterase; MATE, multidrug and toxin extrusion protein; MRP, multidrug resistance–associated protein; NA, not applicable; NTCP, sodium taurocholate–cotransporting polypeptide; OAT, organic anion transporter; OATP, organic anion–transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein; SULT, sulfotransferase.

[a]Essential system. Inhibition studies in hepatocytes may involve multiple transporters.

[b]These models represent an emerging field and will be refined with time. Expression levels of enzymes and transporters in these models are lower than those in vivo (Speer et al., 2019; Chapron et al., 2020; Kasendra et al., 2020).

Generally, NPDI prediction models are designed for the purpose of evaluating NPs as inhibitors or inducers of drug metabolizing enzymes and transporters rather than predicting exposure to NPs. Because the dose(s) of, and thus exposure to, NP constituents are difficult to standardize, these models provide only a conservative estimate of the magnitude of an interaction to confirm

or rule out potential NPDIs. Thus, the goal of in vitro experiments is to generate robust parameters related to activation and induction (e.g., EC_{50}, maximum inductive effect) or inhibition (e.g., IC_{50}, reversible K_i, time-dependent K_I, k_{inact}) behavior as well as parameters related to clearance (e.g., $t_{1/2}$, clearance). The following recommendations pertain to selecting concentration ranges for such experiments.

Prior to isolation of individual NP precipitant constituents, in vitro testing with crude fractions derived during bioactivity-directed fractionation is recommended. Concentrations used for initial bioactivity screening may vary because of differences in extraction methods and assay methodology. Based on the NaPDI Center investigators' collective experience, relatively higher concentrations of the extracts may be needed to identify potential pharmacokinetic interactions mediated by UDP-glucuronosyltransferases (UGTs) compared with the CYPs. For example, cranberry extracts/fractions at 5 and 50 μg/ml showed concentration-dependent inhibition of intestinal CYP3A activity (Kim et al., 2011), and silymarin at 5 and 50 μg/ml showed similar percentage inhibition toward CYP3A and UGT activities (Brantley et al., 2013; Gufford et al., 2014), whereas higher concentrations (20, 60, and 180 μg/ml) were needed to produce concentration-dependent inhibition of UGTs by green tea extracts/fractions (Tian et al., 2018). These concentration ranges can be used to test for reversible inhibition as well as time-dependent CYP inhibition (e.g., based on structural alerts). NADPH or another relevant cofactor (e.g., UDP glucuronic acid) and substrate, respectively, should be used to initiate these reactions.

When testing isolated bioactive constituents, the concentration range should span a pharmacologically relevant concentration of individual constituents (i.e., maximum unbound plasma concentration) and a 10-fold higher concentration. If human plasma concentrations of a given constituent are not available, simulated unbound gut concentrations, simulated unbound hepatic portal venous inlet concentrations, and concentrations approaching constituent solubility can provide initial estimates of the concentrations to be tested (Tian et al., 2018; Cox et al., 2019). Three concentrations of constituents (e.g., 1, 10, and 100 μM) are recommended during initial screening to assess potential concentration-dependent alteration in enzyme/transporter activity. Depending on the results, this concentration range can be adjusted accordingly or used to guide determination of induction (e.g., EC_{50}), reversible inhibition (e.g., IC_{50}, K_i), and/or time-dependent inhibition (e.g., IC_{50} shift, K_I, k_{inact}) potency.

III. Applying or Developing Static and Physiologically Based Pharmacokinetic Models

There are two major categories of modeling strategies that are applicable to different pharmacokinetic NPDI scenarios. Static models refer to those that generate the estimated change in a pharmacokinetic endpoint of the object drug (typically AUC) in the presence of a single concentration of one or more NP constituents. Unless the NP is administered to steady state as an intravenous infusion, the plasma (or gut) concentration of the constituent causing the NPDI will change with time. Dynamic models, such as PBPK models, are capable of incorporating these changing concentrations to predict NPDIs. Such models are used with increasing frequency in the academic, regulatory, and commercial sectors to characterize and simulate DDIs. Both techniques have been used successfully to predict NPDIs involving curcumin and constituents of St. John's wort and milk thistle (Table 3). Publications using PBPK modeling have proliferated approximately 4-fold since 2011, and the FDA has released 24 rule-making and guidance documents on this topic (Kola and Landis, 2004; Tan et al., 2018).

Selection of a static model to predict NPDI risk is a conservative approach. If the NP is a potent inhibitor that results in maximum inhibition of the enzyme/transporter at all plasma or gut concentrations of the NP constituent, then the static and PBPK models will yield identical predictions. Static models that estimate fold changes in object drug AUC have been used to predict pharmacokinetic NPDIs (Zhou et al., 2004, 2005; Brantley et al., 2013; Ainslie et al., 2014; Gufford et al., 2015b; Tian et al., 2018; Bansal et al., 2020; Espiritu et al., 2020; McDonald et al., 2020). In contrast, PBPK models incorporate systems of differential equations to predict the time course of plasma concentrations of both object drug and precipitant NP constituent(s) using an array of in vitro data and a sequence of physiologic compartments (e.g., intestine and liver) in which distribution of the object drug/NP constituent is governed by blood flow, protein binding, and influx and efflux processes, and elimination is governed by blood flow, protein binding, and the intrinsic clearance of metabolic or excretory processes.

A. Developing Pharmacologically Based Pharmacokinetic Models for Natural Product–Drug Interaction Prediction

Few PBPK models for estimating the extent of NPDIs have been reported, although PBPK modeling strategies have been used successfully to predict drug interactions involving silibinin (Brantley *et al.*, 2014b; Gufford *et al.*, 2015a), *Schisandra sphenanthera* (Adiwidjaja et al., 2020b), and St. John's wort (Adiwidjaja et al., 2019). Historically, PBPK modeling was a niche skill that involved solving systems of differential equations, often with manually coded programs. The general structure of a PBPK model is illustrated conceptually (Fig. 2).

Strategies for developing PBPK models depend on the available data and can be bottom-up, top-down, or middle-out. Various platforms have been used to

TABLE 3
Examples of natural product–drug interactions predicted using static and PBPK models

Natural Product			Object Drug(s)	Biochemical Target(s)	Model Type	Change in Object-Drug AUC or R_2		Reference(s)
Common Name	Latin Name	Precipitant Constituent(s)				Predicted	Observed	
Cannabis, marijuana	*Cannabis sativa* L.	CBD, THC	Phenacetin, diclofenac, omeprazole, dextromethorphan, testosterone	CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A	Static	CBD: >5-fold ↑ for CYP2C19 and CYP3A substrates; THC: >5-fold ↑ for CYP2C9 substrates	NR	Bansal et al. (2020)
Cinnamon	*Cinnamomum* spp.	*trans*-Cinnamic aldehyde	Letrozole, nicotine	CYP2A6	Static	1.1- to 3.6-fold ↑	NR	Chan et al. (2016)
		trans-Cinnamic aldehyde, 2-methoxycinnamaldehyde				~4- to 5-fold ↑		Espiritu et al. (2020)
Curcumin (as a solid lipid nanoparticle)	*Curcuma longa* L.	Curcumin, curcumin glucuronide	Imatinib, bosutinib, paclitaxel	CYP2C8, CYP3A4	PBPK	≤1.10-Fold ↑ for imatinib and bosutinib	NR	Adiwidjaja et al. (2020a)
Goldenseal	*Hydrastis canadensis* L.	Berberine, (-)-β-hydrastine, hydrastinine	Dextromethorphan, midazolam, diclofenac	CYP2C9, CYP2D6, CYP3A	Static	R_2 = 1.00, 7.90, and 1.26 for berberine and CYP2C9, CYP2D6, and CYP3A4, respectively; R_2 = 8.94, ~4, and 17.8 for (-)-β-hydrastine and CYP2C9, CYP2D6, and CYP3A4, respectively	NR for dextromethorphan[a] 1.62-fold ↑ for midazolam after 2 wk of goldenseal administration (1323 mg three times daily) NR for diclofenac	Gurley et al., (2008); Guo et al. (2012); McDonald et al. (2020)
Grapefruit juice	*Citrus* × *paradisi* Macfad.	6′,7′-Dihydroxy-bergamottin	Loperamide	CYP3A	Static	1.6-Fold ↑	1.7-fold ↑	Ainslie et al. (2014)
Green tea	*Camellia sinensis* (L.) Kuntze	ECG, EGCG	Raloxifene	UGTs	Static	6.1- and 1.3-fold ↑, respectively, based on estimated concentrations in intestinal lumen and enterocyte	30% ↓	Tian et al. (2018b); Judson et al. (2020)
Kratom	*Mitragyna speciosa* (Korth.) Havil.	Mitragynine	Diclofenac, dextromethorphan, midazolam	CYP2C9, CYP2D6, CYP3A	Static	1.1- and 5.7-fold ↑ for dextromethorphan and midazolam, respectively	NR	Tanna et al. (2020)
St. John's wort	*Hypericum perforatum* L.	Hyperforin[b]	Alprazolam, carbamazepine, docetaxel, ethinyl estradiol, imatinib, midazolam, tacrolimus, verapamil, zolpidem, ibuprofen, tolbutamide, *S*-warfarin, clopidogrel, omeprazole	CYP2C19, CYP2C9, CYP3A	PBPK	2%–79% ↓ for all substrates except clopidogrel (1.31-fold ↑) and *S*-warfarin (1.06-fold ↑).	Close agreement with observed changes (18%–23% difference)	Adiwidjaja et al. (2019)
Silibinin[b]	*Silybum marianum* (L.) gaertn.	Silybin A, silybin B	Midazolam, warfarin	CYP3A, CYP2C9	PBPK	1.05- and 1.04-fold ↑ for midazolam and *S*-warfarin, respectively	1.09 and 1.13-fold ↑ for midazolam and *S*-warfarin, respectively	Brantley et al. (2014b)
Silymarin	*Silybum marianum* (L.) gaertn.	Silybin A, silybin B, isosilybin A, isosilybin B, silychristin	Midazolam	CYP3A	Static	1.75-fold ↑	NR	Brantley et al. (2013)
Silibinin	*Silybum marianum* (L.) gaertn.	Silybin A, silybin B	Raloxifene	UGTs	PBPK	Up to 1.3-fold ↑	1.09-fold ↑	Gufford et al. (2015a)
Silibinin, silymarin	NA, *Silybum marianum* (L.) gaertn.	Silybin A, silybin B	Raloxifene	UGT1A1, UGT1A8, UGT1A10	Static	4- to 5-fold ↑ by silibinin and silymarin, respectively	1.09-fold ↑	Gufford et al. (2015a,b)

CBD, cannabidiol; CYP, cytochrome P450; EGCG, epigallocatechin gallate; NA, not applicable; NR, not reported; R_2, intrinsic clearance ratio (without:with a time-dependent inhibitor); THC, tetrahydrocannabinol; UGT, UDP-glucuronosyltransferase.

[a]9-Fold ↑ in urinary dextromethorphan:dextrorphan after 2 wk of goldenseal administration (300 mg three times daily).

[b]Semipurified extract of milk thistle containing silybin A and silybin B in approximately an equimolar ratio.

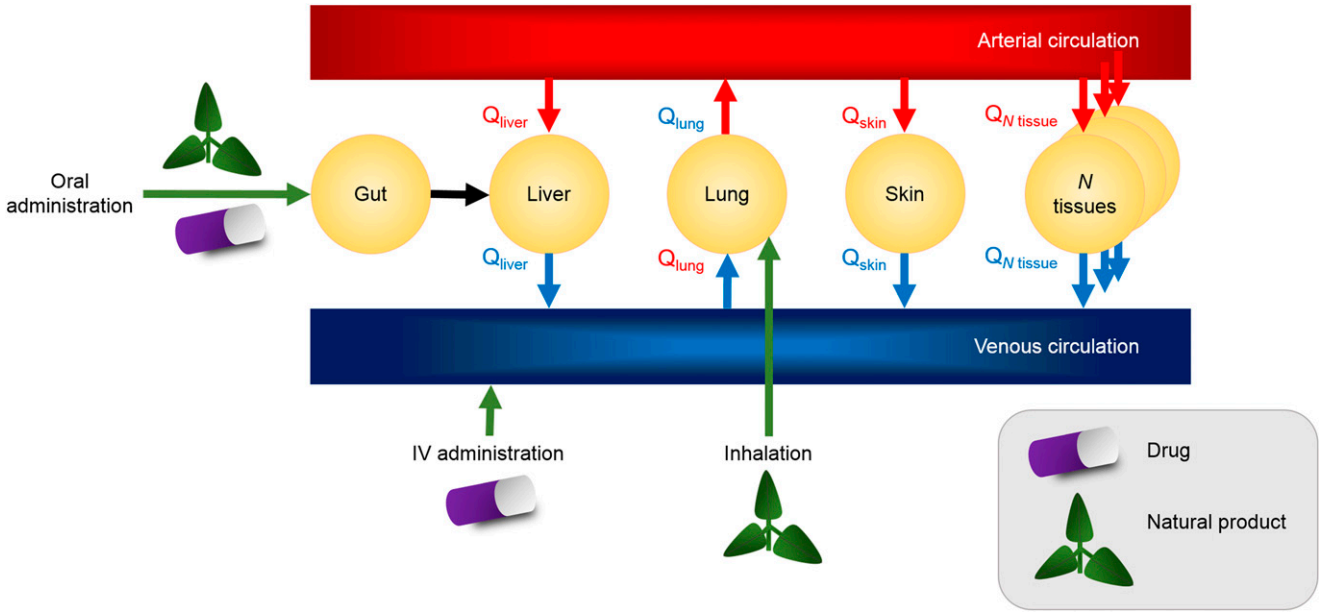


Fig. 2. General structure of a physiologically-based pharmacokinetic model designed to evaluate a natural product–drug interaction. Intravenous administration is rarely, if ever, used for natural products; rather, common routes include oral consumption and inhalation. The number of tissue compartments is variable, but *N* compartments can be included in a full physiologically-based pharmacokinetic model. Input and output blood flow rates (Q) describe constituent passage between the arterial and venous circulation.

construct bottom-up concentration-time prediction models, and differential equation–solving applications have proven to be useful tools for developing PBPK models (Allen, 1990; Lu et al., 2016). When some in vivo data are available, a middle-out approach that integrates existing in vivo and in vitro data can be used to refine uncertain or unknown parameters in the PBPK model; the advantage of this approach is that the model is informed by limited in vivo data (Tsamandouras et al., 2015). Finally, when complete clinical pharmacokinetic data are available, top-down models can be constructed to estimate organ exposures, although these models usually require the assumption of homogenous distribution.

Each modeling strategy requires assumptions (e.g., the expression and abundance of tissue-specific enzymes and transporters). Tutorials and reviews for building these models are available (Sager et al., 2015; Kuepfer et al., 2016). Thus, the scope of this recommended approach is to tailor these recommendations for building PBPK models for NPs and NPDIs.

B. Natural Product Dose Selection

As mentioned earlier, dose estimation is difficult for NPs. Currently, no database exists to collate information on the relative proportions of individual constituents in commercially available NPs. In addition, estimating average consumer NP doses is difficult because NP formulations vary widely between manufacturers, lots, and batches, and NP standardization is relatively nonexistent (Brantley et al., 2014a; Paine et al., 2018). For NPs administered as an aqueous solution (e.g., flavonoids in grapefruit juice), the dose can be approximated as the quantity of constituent in the volume of a glass of juice (e.g., 250 ml) (Johnson et al., 2017). The lack of standardized NP doses necessitates a sensitivity analysis with varying doses to predict the magnitude range for an NPDI.

C. Modeling Using Commercial Applications

Commercially available software platforms are designed to require minimal input from the end user and typically run full PBPK models that operate on systems of differential equations governing dissolution, solubility, absorption, distribution, metabolism, and excretion. An advantage of these platforms is the ability to simulate populations with large intersubject variation (e.g., by Monte Carlo methods) in these determinants of xenobiotic disposition. Additionally, effects of age, sex, race, and physiologic conditions, such as disease and pregnancy, on xenobiotic disposition can be simulated using commercial software.

Because manual entry of physiologic model parameters and equations is not required, end users may run simulations without changing input parameters. At minimum, the default software settings should be carefully evaluated, and all input values and settings should be reported. Commercial applications typically include a library of default object drugs. These drugs should be carefully evaluated to ensure that the correct object drugs are selected according to published guidelines (Fuhr et al., 2019).

IV. Building Physiologically Based Pharmacokinetic Models De Novo for NPDIs

Unlike PBPK models developed using commercial software, PBPK models developed de novo provide full

control over model characteristics. Design considerations are described below.

A. Compartments and Parameterization

The degree of complexity used in a PBPK model can vary from minimal (e.g., a three-compartment model) to high (e.g., a model with many physiologic compartments) (Sager et al., 2015). A full PBPK model can produce concentration-versus-time estimates in many physiologic compartments, potentially providing greater insight into the mechanism of an NPDI. However, the potential increase in accuracy from a more compartmentalized model can be achieved only if the necessary physiologic parameters (blood flow, organ composition) and NP physicochemical parameters (e.g., tissue partition coefficient, pK_a) are available. Complicated dissolution and absorption models may improve model performance but can be implemented only if the necessary physicochemical and in vitro data are available.

B. Verification

PBPK models can be built manually as systems of differential equations or generated using machine-learning approaches. Regardless of the approach, a separate verification data set should be used for final assessment of model prediction accuracy. Acceptable prediction accuracy should be specified before conducting PBPK modeling and simulation.

C. Error Checking

To avoid physiology-related errors while parameterizing models, checkpoints should be used to ensure physiologic relevance (e.g., the sum of blood flows should be equivalent to the expected cardiac output scaled for a human of certain age and sex). Sources of these reference values may include curated databases, such as those maintained by the US Environmental Protection Agency for PBPK modeling (https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=204443). Evaluating models in alternate programming languages and/or with independent datasets provides an additional layer of model verification and quality assurance. When possible, comparing a de novo model to that developed using a commercial program may provide insight into critical differences in predicted pharmacokinetic endpoints (Gufford et al., 2015a).

D. Reporting

Reproduction of a PBPK model is impossible without comprehensive reporting of model characteristics. Ideally, the complete code for a custom PBPK model should be published or made available for purposes of reproduction (Sager et al., 2015). Likewise, all inputs for a PBPK model developed using commercial software should be provided. Ensuring the availability of the relevant information is incumbent on both the editors and reviewers of relevant journals.

V. Using Static and Physiologically Based Pharmacokinetic Models to Prioritize Natural Product–Drug Interaction Risk

The NaPDI Center posits that NPDIs should be evaluated with at least the same level of rigor as that mandated for DDIs (FDA, 2020). Thus, a sequential set of decision trees are proposed to guide decision-making (Fig. 3).

A. Initial Assessment of Natural Product–Drug Interaction Risk

Investment of time and computing resources into development of complex PBPK models is not necessary for every NP constituent. Rather, simple initial assessments should be conducted to determine which constituent(s) may merit modeling studies.

For rapid triage of multiple NP constituents, predicted physicochemical properties can be used to populate commercial modeling software programs that include the target tissue as a compartment. Estimated concentrations at the tissue site(s) of interest can then be compared with reported inductive or inhibitory concentrations from in vitro experiments. If the predicted maximum unbound plasma concentration of the NP constituent(s) is within 10% of (FDA, 2020) or exceeds the in vitro unbound inductive or inhibitory concentration (e.g., unbound concentration at half maximum inductive effect, unbound IC_{50}, $K_{i,u}$), then PBPK modeling of the NPDI is warranted. Alternatively, if the target drug metabolizing enzyme or transporter is pharmacologically important in the gut (e.g., CYP3A or organic anion–transporting polypeptide 2B1) (Won et al., 2010; 2012) and the gut tissue/luminal concentration estimated by the modeling approach is near or exceeds the unbound inductive or inhibitory concentration, then static and PBPK models should be used to predict the likelihood and magnitude of an NPDI (FDA, 2020). A decision process for developing PBPK models of NPDIs is presented (Fig. 3; Table 4).

VI. Future Research

As for DDIs, if a clinically significant pharmacokinetic NPDI is suspected, the interaction merits advancement to a clinical study. The design of such a study is critical and will be addressed in a separate recommended approach from the NaPDI Center.

A. Natural Product–Drug Interactions within the Gastrointestinal Tract

Precision in modeling NPDIs mediated by drug metabolizing enzymes and transporters expressed in the intestine is governed primarily by the difficulty in predicting intracellular unbound concentrations of absorbed and effluxed NP constituents. Because intestinal epithelial cells polarize into an apical (brush

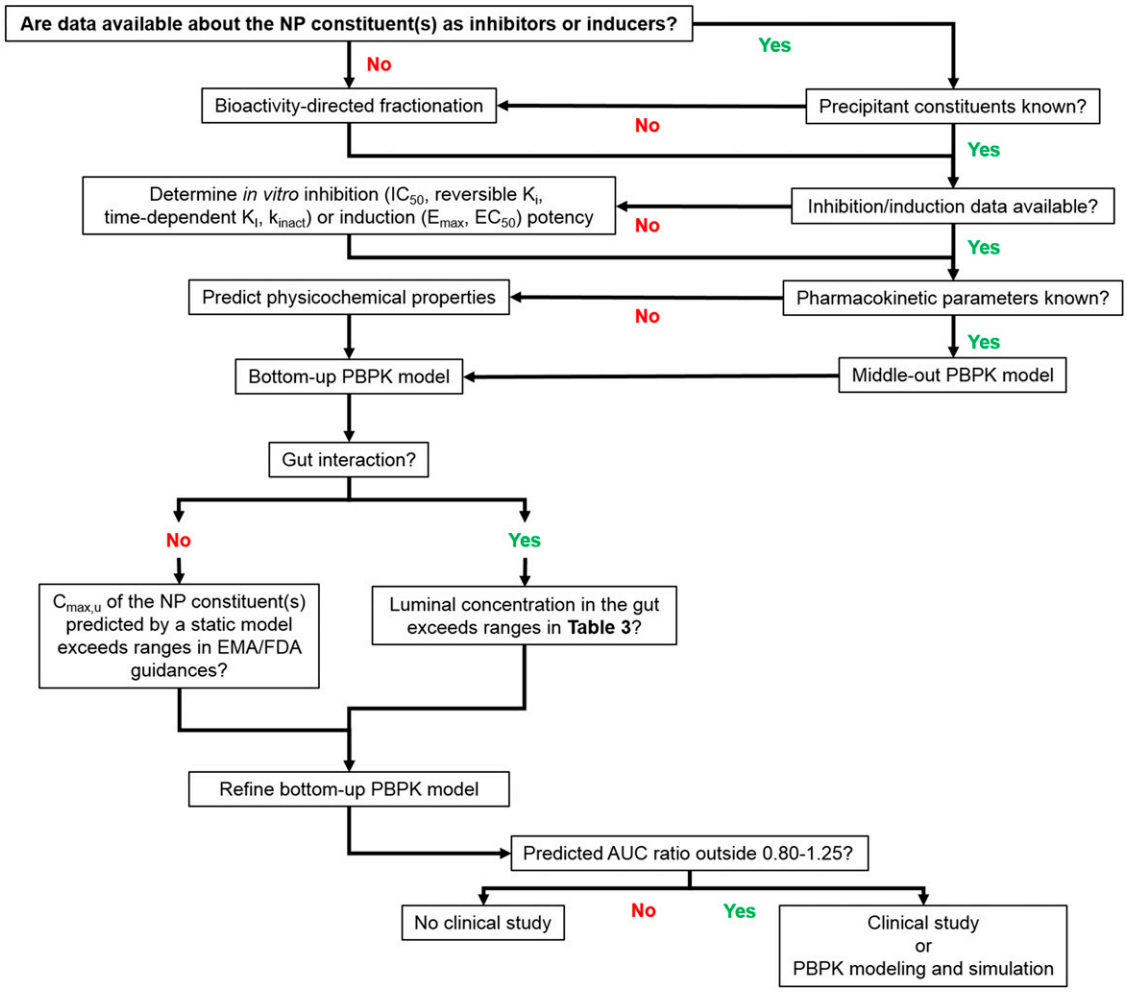


Fig. 3. Decision tree for the development of PBPK models of natural product–drug interactions. Selection of a modeling strategy depends on the available data. If data about the induction and inhibition behavior of the natural product constituent(s) are not available in the literature, these data can be gathered from in vitro experiments. If the predicted concentrations of the constituent(s) in either the gut or the plasma exceed the cutoffs [Table 4 and FDA and European Medicines Agency (EMA) guidance], different types of modeling are warranted. $C_{max,u}$, maximum unbound concentration; E_{max}, maximum inductive effect; k_{deg}, degradation rate constant; K_I, inhibitor concentration at one-half maximum inactivation rate; k_{obs}, inactivation rate constant (observed).

border) and a basolateral domain, intestinal transporters show orientation-related expression. Thus, the extent of an NPDI mediated by an intestinal transporter should be driven by the local intracellular (for efflux transporters) or extracellular (for uptake transporters) concentration of the NP constituent at the membrane (apical or basolateral) where the transporter is expressed (Fig. 4). These concentrations may be

TABLE 4
Gut-specific cutoffs or criteria for natural product–drug interactions

Cutoffs or criteria used in decision tree for physiologically-based pharmacokinetic modeling of natural product–drug interactions depicted in Fig. 3. For additional information or for cutoff values related to other organ systems, refer to (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf; FDA, 2020).

Transporter Inhibition		
P-gp and BCRP	$(Dose/250\ ml)/K_{i,u}$ (or $IC_{50,u}$) ≥ 10	
Enzyme Inhibition		
	Reversible Inhibition	Time-Dependent Inhibition
CYP3A	$(Dose/250\ ml)/K_{i,u} \geq 10$	$k_{obs}/k_{deg} \geq 10$, wherein $k_{obs} = (k_{inact} \cdot Dose/250\ ml)/(K_{I,u} + Dose/250\ ml)$
CYP Induction[a]		
1. Concentration-dependent increase in mRNA expression of a CYP 2. ≥ 2-Fold increase of CYP mRNA expression relative to vehicle control at expected gut drug concentrations 3. Increase ≥20% of the positive control response		

BCRP, breast cancer resistance protein; CYP, cytochrome P450; $IC_{50,u}$, unbound IC_{50}; k_{deg}, degradation rate constant; $K_{i,u}$, unbound reversible inhibition constant; k_{obs}, inactivation rate constant (observed); P-gp, P-glycoprotein.

[a]Must satisfy all three criteria to qualify as a CYP inducer. Criteria are based on those recommended for hepatic CYP induction (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf; FDA, 2020).

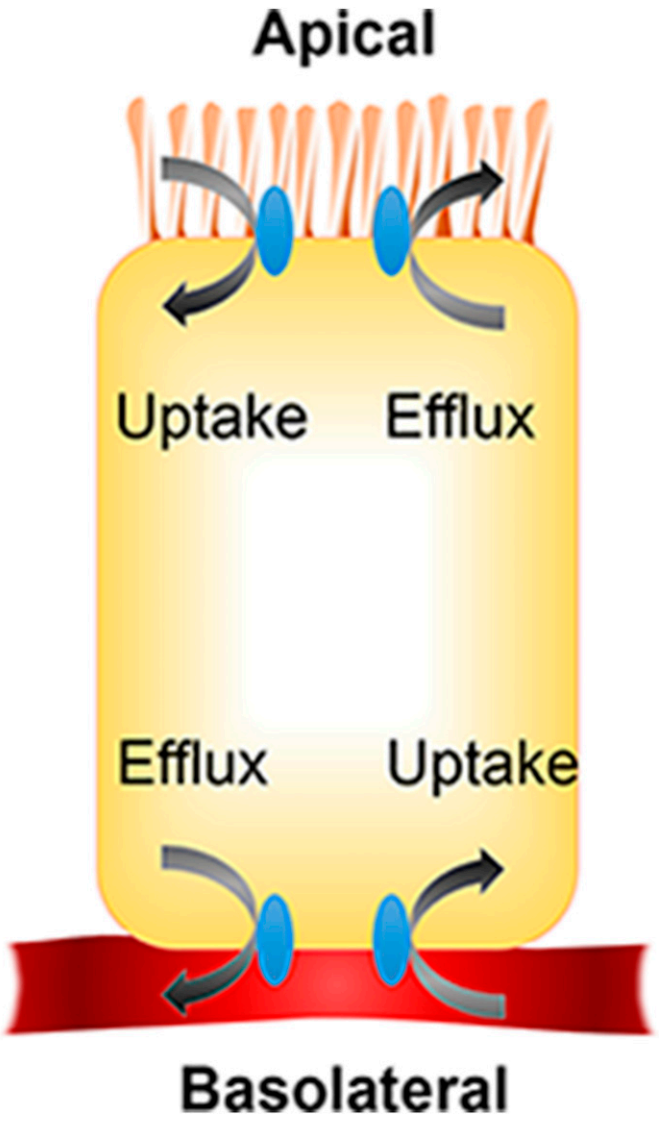


Fig. 4. Illustration of intestinal cell polarization and the relative orientations of uptake and efflux transporters.

reasonably approximated by the concentration of an NP constituent in the intestinal lumen. For example, for uptake transporters expressed on the apical membrane, unbound intestinal lumen concentrations would be the driving force. Further complicating these calculations is the unstirred water layer covering the apical membrane of enterocytes, which effectively constitutes an aqueous barrier to absorption both in vitro and in vivo (Korjamo et al., 2008; Wood et al., 2018). For an intracellular enzyme or efflux transporter expressed on the basolateral membrane of enterocytes, the intracellular unbound concentration would be more relevant, with the intestinal lumen concentration serving as a driver of intracellular concentration during the absorptive phase.

Another area of future research for PBPK modeling of NPDIs relates to the impact of the gut microbiota on plasma and target tissue exposure to object drugs. Gut microbiota can contribute to prodrug activation (e.g., the sulphanilamide-generating prodrugs prontosil and neoprontosil and the 5-aminosalisylic acid–generating prodrugs sulfasalazine, balsazide, and osalazine) (Wilson and Nicholson, 2017). Additionally, the gut flora directly execute a number of drug metabolic reactions, including decarboxylation, demethylation, hydrolysis, and dehydration (Wilson and Nicholson, 2017; Clarke et al., 2019). There is also emerging evidence that the secretory gut flora metabolome can alter drug metabolizing enzyme and transporter expression in the gut and liver (Fu and Cui, 2017; Nichols et al., 2019) and the drug molecules on which they act. Thus, there may be NPDIs mediated by gut microbiota. The contribution of the gut flora to NPDIs is a largely untapped area of future research.

B. Natural Product Metabolites

Currently, for NCEs, evaluation of a metabolite as a substrate and inducer/inhibitor of drug metabolizing enzymes and transporters is warranted if a metabolite is 1) less polar and exhibits at least 25% of the AUC compared with the parent or 2) more polar and has equal or greater AUC compared with the parent (FDA, 2020).

For NP phytoconstituents, metabolite data are often not available, raising concerns about the risk of unidentified NPDIs. NP phytoconstituents can undergo significant first-pass metabolism in the gut and liver, generating quantitatively major circulating products with uncharacterized effects on pharmacokinetic processes, as well as reactive metabolites that inactivate the enzymes that produce them. The recent development of the biochemometric approach discussed above may identify NP constituent metabolites that are precipitants of NPDIs. However, such examples have yet to be reported.

C. Systems Biology

Another logical step for improving the understanding of pharmacokinetic NPDIs is to integrate systems biology models with PBPK models. One systems biology tool potentially helpful to NPDI research is the virtual metabolic human database (Noronha et al., 2019). This recently developed database connects human metabolism with genetics, human-associated microbial metabolism, nutrition, and diseases. The use of -omics tools and the virtual human metabolic database have yet to be explored for NPDIs but may eventually offer unique mechanistic insight that can contribute to PBPK modeling.

VII. Conclusions

The application of static and PBPK models to potential NPDIs may allow rapid and systematic assessment of NPDI risk. Given the breadth and popularity of the NP consumer market, the lack of strict regulation on NPs with high NPDI risk, and the cost and time

associated with conducting clinical studies, mathematical modeling provides a plausible method for mitigating the public health risk of NPDIs. Widespread adoption of systematic approaches to NPDI model development and application will facilitate the identification and investigation of NPDIs and promote the dissemination of critical NPDI information to researchers, clinicians, and patients.

Acknowledgments

The authors thank Nicholas Oberlies and Preston Manwill (University of North Carolina Greensboro, Greensboro, NC) for confirming the Latin names of the natural products listed in Table 3. M.F.P. dedicates this article to David P. Paine.

Authorship Contributions

Performed data analysis: Cox, Tian, Paine.

Wrote or contributed to the writing of the manuscript: Cox, Tian, Clarke, Rettie, Unadkat, Thummel, McCune, Paine.

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