Expert Review

The Role of Transporters in the Pharmacokinetics of Orally Administered Drugs

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Abstract. Drug transporters are recognized as key players in the processes of drug absorption, distribution, metabolism, and elimination. The localization of uptake and efflux transporters in organs responsible for drug biotransformation and excretion gives transporter proteins a unique gatekeeper function in controlling drug access to metabolizing enzymes and excretory pathways. This review seeks to discuss the influence intestinal and hepatic drug transporters have on pharmacokinetic parameters, including bioavailability, exposure, clearance, volume of distribution, and half-life, for orally dosed drugs. This review also describes in detail the Biopharmaceutics Drug Disposition Classification System (BDDCS) and explains how many of the effects drug transporters exert on oral drug pharmacokinetic parameters can be predicted by this classification scheme.

KEY WORDS: BDDCS; drug transporters; hepatic; intestinal; transporter/enzyme interplay.

INTRODUCTION

Since the discovery of Pgp in 1976 (1), the importance of drug transporters in influencing the pharmacokinetics of orally dosed drugs has become increasingly evident (2–4). Drug transporters in both the gut and the liver can help control access of drugs to systemic circulation by dictating the amount of drug that enters the body from the gut lumen and influencing how much drug escapes first pass metabolism in both gut and liver. The Biopharmaceutics Drug Distribution and Classification System (BDDCS) can be a helpful tool in predicting the effects that drug transporters in the gut and liver will have on a drug's pharmacokinetic profile (5). The BDDCS is a modification of the Biopharmaceutics Classification System (BCS) proposed by Amidon *et al.* (6) that is based on the permeability and solubility characteristics of a drug compound. This review seeks to briefly discuss intestinal and hepatic transporters, review the BDDCS, summarize the effects of transporters in both the gut and the liver on pharmacokinetic parameters of orally dosed drugs, and seek how to potentially predict some of these effects using the BDDCS.

TRANSPORTERS

A large amount of work has identified and characterized intestinal and hepatic transporters in regards to tissue expression profiles, regulation, mechanisms of transport, substrate and inhibitor profiles, species differences, and genetic polymorphisms. A detailed review of this work is beyond the scope of this article, but further information can be found in several recent extensive reviews (7–20).

Both uptake and efflux transporters are important in determining oral drug disposition by controlling absorption and bioavailability. See Figs. 1 and 2 for their localization in enterocytes and hepatocytes and Table I for examples of substrates for those transporters expressed in the intestine and/or liver. The major uptake transporters responsible for xenobiotic transport belong to the two solute carrier (SLC and SLCO) superfamilies. The SLC superfamily members typically transport Type I organic anions (21), cations, and zwitterions (22), while the SLCO superfamily transports Type II organic anions (21). Members of the SLC and SLCO families use a variety of porter mechanisms (i.e. uniporter, antiporter, symporter), not all of which have been fully elucidated for each specific transporter. In general, these transporters do not utilize an energy source other than a chemiosmotic gradient created by translocation of ions across the membrane (7,9,23–25). The SLC superfamily encompasses a variety of transporters, including the organic anion transporters (OAT, SLC22A), the organic cation transporters (OCT, SLC22A), the electroneutral organic cation transporters (OCTN, SLC22A), the equilibrative nucleoside transporters (ENT, SLC29), the concentrative nucleoside transporters (CNT, SLC28), the apical Na$^+$−dependent bile salt transporter (ASBT, SLC10), the monocarboxylate transporters (MCT, SLC16), and the peptide transporters (PEPT, SLC15). The SLCO family is made up of the organic anion transporting polypeptides (OATP).

Efflux transporters expressed in the intestine and liver include P-glycoprotein (Pgp, ABCB1), bile salt export pump (BSEP, ABCB11), multidrug resistance proteins (MRP1- 6, ABCC1-6), and breast cancer resistance protein (BCRP, ABCG2), all members of the ATP-Binding Cassette super-family (14,26). Members of this superfamily use ATP as an

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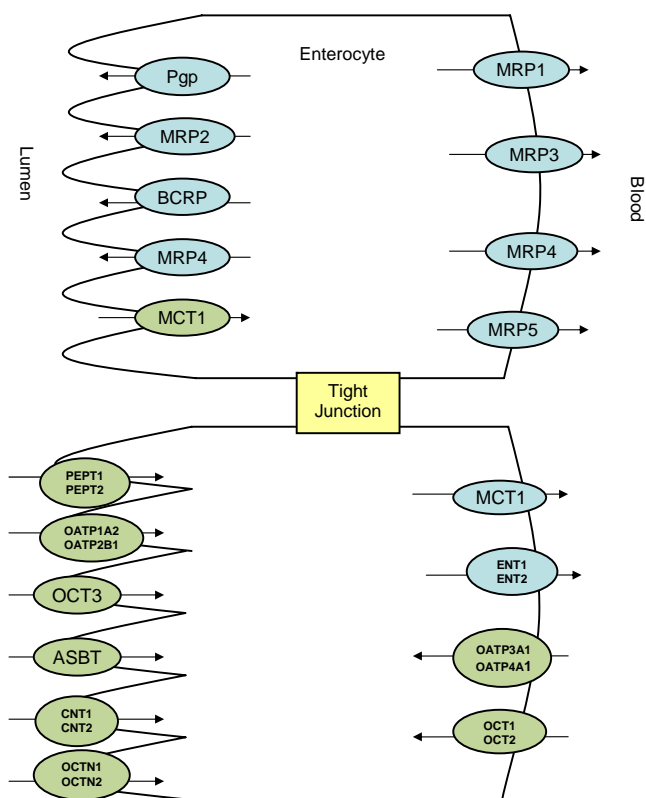
Fig. 1. Enterocyte with intestinal uptake (green) and efflux (blue) transporters. Multidrug resistance protein (MDR), Multidrug resistance associated protein (MRP), Breast cancer resistance protein (BCRP), Monocarboxylate transporter protein (MCT), Peptide transport protein (PEPT), Organic anion transporting polypeptide (OATP), Organic cation transporter (OCT), apical sodium-dependent bile acid transporter (ASBT), Concentrative nucleotide transporter (CNT), Electroneutral organic cation transporter (OCTN), Equilibrative nucleoside transporter (ENT). Adapted from Custodio *et al.* (58).

energy source, allowing them to pump substrates against a concentration gradient.

These transporters' expression in the intestine and/or liver, the two major sites affecting how much drug will get into the body after an oral dose, makes factors affecting their function important determinants of oral drug pharmacokinetics. Regulatory elements controlling their protein levels, genetic polymorphisms leading to increased or reduced function, and coadministration with inhibitors are all important avenues by which a transporter's ability to transport substrates is altered. While an extensive discussion of these factors is not the purpose of this review, Tables I, II, and III list commonly used transporter inhibitors, established polymorphisms shown to have an effect in human clinical studies, and known regulatory elements for some transporters.

THE BIOPHARMACEUTICS DRUG DISPOSITION CLASSIFICATION SYSTEM (BDDCS)

The BDDCS (5) is a modification of the BCS (6), in which compounds are categorized in four classes according to their permeability and solubility, characteristics that will greatly influence oral absorption. The BCS (See Fig. 3) was developed to aid in predicting *in vivo* pharmacokinetic

behavior from *in vitro* solubility and *in vitro* or *in vivo* permeability measurements. The FDA, hoping to ease regulatory and financial burdens, enacted the BCS as a method for determining which compounds may receive a waiver for *in vivo* bioavailability and bioequivalence testing (27).

Class 1 compounds, having high solubility and permeability, formulated as immediate release solid dosage forms that display rapid dissolution may be eligible for the waiver. Solubility is relatively easily determined; however this is not the case with permeability. The FDA, in implementing the BCS, considers a highly permeable compound as one that achieves 90% absorption in humans (27). This is based on the finding that all of the compounds initially studied in human *in situ* gut permeability studies (28,29) exhibiting permeability equal to or greater than metoprolol (1.34×10^{-4} cm/sec; 17 of 29 compounds) show equal or more than 90% extent of absorption in humans. In contrast, those 12 compounds exhibiting permeability less than metoprolol were less than 90% absorbed in humans. This is an unfortunate coincidence with the potential for confusing applicability, since permeability is a rate parameter, whereas percent absorption is an extent parameter, and they are not interchangeable. A situation can be envisioned in which a compound has low permeability but achieves high absorption. For example, sotalol exhibits a low P_{app} in the apical to basolateral direction of 1.53 ± 0.29 ($\times 10^{-6}$ cm/sec) in a Caco2 cell line yet has 95% absorption compared to other compounds with similar P_{app} values that show only 50% (atenolol) and 70% (acebutolol) absorption (30).

The recognition that BCS Class 1 and 2 compounds are mainly eliminated by metabolism while Class 3 and 4 compounds are mainly eliminated by biliary or renal excretion of unchanged drug led to the development of the BDDCS (See Fig. 4 and Table IV) by Wu and Benet (5), in which the permeability characteristic was replaced by route of elimination. It is believed that extent of metabolism (by oxidative Phase 1 and conjugative Phase 2 enzymes) is an appropriate surrogate for permeability since for a drug to get metabolized, it must get absorbed (31). Generally, a drug's
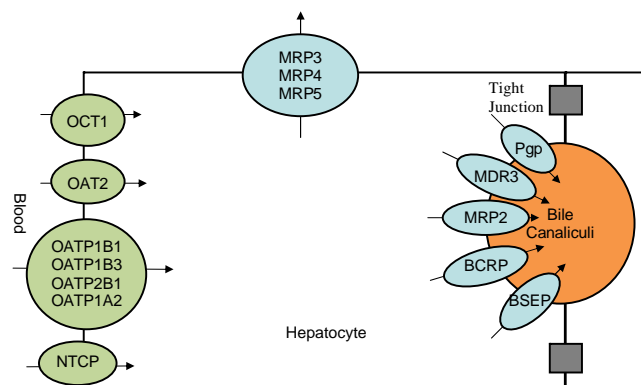

Fig. 2. Hepatocyte with hepatic uptake (green) and efflux (blue) transporters. Multidrug resistance associated protein (MRP), Organic cation transporter (OCT), Organic anion transporter (OAT), Organic anion transporting polypeptide (OATP), sodium-taurocholate cotransporting polypeptide (NTCP), P-glycoprotein (Pgp), Multidrug resistance protein (MDR), Breast cancer resistance protein (BCRP), Bile salt export pump (BSEP). Adapted from Custodio *et al.* (58).

Table I. Substrates and Inhibitors of Intestinal and Hepatic Transporters

	Substrates	Inhibitors
Gut/Apical Gene (Protein)		
SLC16A1 (MCT1)	lactate, pyruvate, benzoic acid (96)	AR-C117977 (111)
SLC15A1 (PEPT1)	ampicillin, captopril, valacyclovir (97)	4-aminomethylbenzoic acid, Lys[Z(NO(2))]-Pro (112,113)
SLC15A2 (PEPT2)	ampicillin, captopril, valacyclovir (97)	cefadroxil, captopril (114)
SLCO2B1 (OATP2B1)	estrone-3-sulfate, atorvastatin (98)	rifampin, bromosulfophthalein (97)
SLCO1A2 (OATP1A2)	estrone-3-sulfate, deltorphin II, fexofenadine (99)	rifampin, bromosulfophthalein (97)
SLC22A3 (OCT3)	cimetidine (97)	corticosterone, decynium22 (115)
SLC10A2 (ASBT)	bile salts (taurocholate) (100)	S-8921, SC-435 (116,117)
SLC28A1 (CNT1)	thymidine, zidovudine, zalcitabine (101)	adenosine (118)
SLC28A2 (CNT2)	guanosine, ribavirin (102)	thymidine (119)
SLC22A4 (OCTN1)	quinidine, verapamil (97)	cimetidine, pyrilimine, verapamil (120)
SLC22A5 (OCTN2)	quinidine, verapamil (97)	pyrilamine, TEA (121)
ABCB1 (Pgp)	digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel (97)	GG918, verapamil (97)
ABCC2 (MRP2)	indinavir, cisplatin, glutathione-, glucuronide-, sulfate-conjugates (97,103)	MK-571, cyclosporine (97,122)
ABCG2 (BCRP)	rosuvastatin, doxorubicin, sulfasalazine, topotecan, daunorubicin (97)	FTC, Ko-143 (123)
ABCC4 (MRP4)	topotecan, ceftizoxime, cefazolin, urate (104,105)	MK-571 (122)
Gut/Basolateral Gene (Protein)		
SLCO3A1 (OATP3A1)	PGE1, PGE2, BQ-123, vasopressin (106)	
SLCO4A1 (OATP4A1)	deesterified unoprostone carboxylate (107)	
SLC22A1 (OCT1)	acyclovir, amantadine, desipramine, ganciclovir, metformin (97)	montelukast (124)
SLC22A2 (OCT2)	amantadine, cimetidine, memantine (97)	bromosulfophthalein (125)
ABCC1 (MRP1)	adefovir, indinavir (97)	MK-571, probenecid (122,126)
ABCC3 (MRP3)	etoposide, methotrexate, tenoposide (97)	MK-571, indomethacin (122,127)
ABCC4 (MRP4)	topotecan, ceftizoxime, cefazolin (105,106)	MK-571 (122)
ABCC5 (MRP5)	cGMP, methotrexate, mercaptopurine (108)	MK-571, sildenafil (122,128)
SLC16A1 (MCT1)	lactate, pyruvate, benzoic acid (96)	AR-C117977 (111)
SLC29A1 (ENT1)	adenosine (109)	nitrobenzylmercaptopurine riboside, dipyridamole (130,131)
SLC29A2 (ENT2)	ddC (2'3'-dideoxycytidine), AZT (3'-azido-3'-deoxythymidine), ddI (2',3'-dideoxyinosine) (110)	nitrobenzylmercaptopurine riboside, dipyridamole (130,131)
Hepatic/Apical Gene (Protein)		
ABCB1 (Pgp)	digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel (97)	GG918, verapamil (97)
ABCB4 (MDR3)	digoxin, paclitaxel, vinblastine (97)	cyclosporine, vinblastine, verapamil (132)
ABCC2 (MRP2)	indinavir, cisplatin, ciprofloxacin, glutathione-, glucuronide-, sulfate-conjugates (97,103)	MK-571, probenecid (122,126)
ABCB11 (BSEP)	vinblastine (97)	cyclosporine, troglitazone (133)
ABCG2 (BCRP)	rosuvastatin, doxorubicin, sulfasalazine, topotecan, daunorubicin (97)	FTC, Ko-143 (123)
Hepatic/Basolateral Gene (Protein)		
SLC22A1 (OCT1)	acyclovir, amantadine, desipramine, ganciclovir, metformin (97)	prazosin, verapamil (134)
SLCO1B1 (OATP1B1)	rifampin, rosuvastatin, methotrexate, pravastatin, thyroxine (97)	rifampin (86,97)
SLCO1B3 (OATP1B3)	digoxin, rifampin, methotrexate (97)	rifampin (86,97)
SLCO2B1 (OATP2B1)	estrone-3-sulfate, atorvastatin (98)	rifampin (86)
SLCO1A2 (OATP1A2)	estrone-3-sulfate, deltorphin II, fexofenadine (99)	rifampin (86)
SLC10A1 (NTCP)	rosuvastatin (97)	bosentan, propranolol, cyclosporine (135,136)
SLC22A7 (OAT2)	zidovudine (97)	pravastatin, probenecid, taurocholate (137,138)
ABCC3 (MRP3)	etoposide, methotrexate, tenoposide (97)	MK-571, indomethacin (122,127)
ABCC4 (MRP4)	topotecan, ceftizoxime, cefazolin (104,105)	MK-571 (122)
ABCC5 (MRP5)	cGMP, methotrexate, mercaptopurine (108)	MK-571, sildenafil (122,128)
ABCC6 (MRP6)	cisplatin, daunorubicin (97)	verapamil, indomethacin, benzbromarone (122,129)

Table II. Regulatory Nuclear Hormone Receptors and Ligands

Gene (Protein)	Nuclear Receptor	Ligand
ABCB1 (MDR1)	PXR, CAR	rifampin (139,140)
ABCC2 (MRP2)	PXR, CAR, FXR	rifampin, phenobarbital, chenodeoxycholic acid (141)
ABCC3 (MRP3)	PXR, CAR	rifampin, phenobarbital (142)
ABCC4 (MRP4)	CAR	rifampin (143)
ABCG2 (BCRP)	PPARg	rosiglitizone (144)
ABCB11(BSEP)	FXR	chenodeoxycholic acid (145)
SLC10A1 (NTCP)	GR	dexamethasone(146)
SLCO1A2 (OATP1A2)	PXR	rifampin (147)
SLCO1B1 (OATP1B1)	PXR	rifampin (142,148)
SLCO1B3 (OATP1B3)	FXR	chenodeoxycholic acid (149)

elimination route is much easier to determine than its extent of absorption or its permeability in humans. Currently, there are several methods for determining drug permeability detailed in the FDA CDER guidance (27). Human pharmacokinetic studies to determine mass balance or absolute bioavailability can be used, but they can be affected by high inter-subject variability and are expensive and time-consuming. Human *in vivo* intestinal perfusion studies, extensively reviewed by Lennernas *et al*. (32), are useful, and several methods are available to conduct these experiments, but they have the same drawbacks as pharmacokinetic studies. Intestinal perfusion studies with representative animal models either *in vivo* or *in situ*, or excised tissue studies with human or animal intestinal tissue are other options, as are *in vitro* cell studies in an epithelial cell line, such as Caco2 monolayers (27). While the use of animal models is less expensive and time-consuming than human studies, there are species differences in expression and substrate profiles for transporters and metabolic enzymes that can lead to misclassification of compounds (33–35). Indeed, the CDER guidance recommends that non-human systems be used only for compounds transported by passive processes (27). When using human cell lines in permeability studies, care must be taken to ensure that expression levels of transporters are representative of those seen *in vivo* (36–38); otherwise, discrepancies could arise between *in vitro* and *in vivo* results.

The use of human liver microsomes, microsomes generated from Sf9 insect cells expressing recombinant metabolic enzymes, and isolated human and animal hepatocytes are widely used to help determine if a drug will be eliminated by metabolism. Simple incubation experiments will determine if a compound is a substrate for metabolizing enzymes and can be followed by *in vivo* animal and human studies to determine the importance of metabolism as a route of elimination. Information on elimination route is obtained by measuring parent drug and metabolite levels in plasma and urine collected during Phase 1 human pharmacokinetic studies.

In the BDDCS framework, if a drug is ≥90% metabolized (a conservative estimate that matches the 90% absorbed

Table III. Examples of Clinically Relevant Polymorphisms

Gene (Protein)	SNP	Haplotype	Clinical Association
ABCB1 (Pgp)	3435C>T		reduced digoxin exposure (150)
	3435C>T		increased digoxin exposure (151)
	2677G>T		increased digoxin exposure (152)
		C3435C>T, 2677G>A	decreased fexofenadine exposure (153)
ABCC2 (MRP2)		-1549G>A, -1019A>G, -24C>T, 1249G>A, IVS26, -34T>C, and 3972C>T	reduced irinotecan-associated diarrhea (154)
ABCG2 (BCRP)	421C>A		reduced diflomotecan bioavailability (155)
	421C>A		increased topotecan bioavailability (156)
	421C>A		decreased rosuvastatin exposure (157)
SLCO1B1 (OATP1B1)		388A>G, 521T>C	increased pravastatin exposure (158)
	521T>C		increased pravastatin exposure (159)
	521T>C		increased fexofenadine exposure (160)
	521T>C		increased repaglinide exposure (161)
	388A>G	388A>G, 521T>C	increased pitavastatin exposure (162)
	521T>C		increased rosuvastatin exposure (163)
	521T>C		increased atorvastatin exposure (163)
	521T>C		increased nateglinide exposure (164)
	521T>C		increased atrasentan exposure (165)
	388A>G	388A>G, 521T>C	increased irinotecan exposure (166)
		388A>G, 521T>C	increased ezetimibe exposure (167)
	521T>C		increased torsemide exposure (168)
SLCO1B3 (OATP1B3)	T334G, G699A		increased mycophenolic acid exposure (169)
SLC22A1 (OCT1)	R61C, G401S, 420del	G465R, 420del; G174S, 420del	increased metformin exposure (65)

High Solubility | Low Solubility

Class 1

High Solubility
High Permeability
Rapid Dissolution

Class 2

Low Solubility
High Permeability

Class 3

High Solubility
Low Permeability

Class 4

Low Solubility
Low Permeability

(High Permeability / Low Permeability on left axis)

Fig. 3. The Biopharmaceutics Classification System (BCS) as put forth by Amidon *et al.* (6) and defined by the FDA (21).

criteria currently used in BCS), then based on solubility, the drug can be designated as Class 1 or 2. The ≥90% absorption of BCS (21) and the recommendation of Benet *et al.* (23) to add ≥90% metabolized as an alternative basis for allowing a waiver of *in vivo* bioequivalence are regulatory issues. Wu and Benet (5) recommend for transporter predictions utilizing ≥70% metabolism for Class 1 and 2 drugs and <70% metabolism for Class 3 and 4 drugs. They point out that in fact very few marketed drugs fall into the 30 to 70% metabolism category and that most drugs are either extensively metabolized (≥70%) or markedly poorly metabolized (<30%).

The main difficulty in utilizing BCS to make predictions about drug disposition, and particularly the effect of transporters, relates to the fact that the FDA has promulgated a regulatory definition of permeability that differs from the scientific definition. The FDA defines a drug as BCS Class 1 when the extent of absorption (a thermodynamic measure) is ≥90%, and then states that this is a highly permeable (a kinetic measure) compound even when measured permeability is low. This ambiguity is reflected in the recent paper of Chen and Yu (39). Our laboratory is in the process of addressing this issue in more detail (40). Talinolol is an excellent example of the BCS/BDDCS difference. Talinolol is listed in all BCS compilations as a Class 2 drug because it has a high extent of absorption. However, its measured permeability is low (41). BDDCS classifies talinolol as a Class 4 drug because it is not metabolized, and thus predicts its poor permeability and it being a substrate for both an uptake and efflux transporter as demonstrated by Shirasaka *et al.* (41).

One of the major misconceptions of the scientific community is that BCS and BDDCS allow prediction of the extent of absorption. Because FDA has confounded permeability and extent of absorption, BCS cannot predict absorption. That is, a drug is considered to be highly absorbed in BCS as a result of the absorption measurement, independent of whether permeability is high or low. In contrast, if a drug is extensively metabolized via Phase 1 and Phase 2 enzymes, BDDCS predicts it will be highly absorbed. However, if a

drug is poorly metabolized, BDDCS provides no predictions of the extent of absorption; it may be high (in some cases because of transporters) or low. BDDCS, however, tells you that transporters must be evaluated.

The BDDCS allows for some general predictions regarding the role of transporters in oral drug disposition based upon the drug's classification (Fig. 5). The relevant transporters in terms of BDDCS class in Fig. 5 reflect what Wu and Benet observed for the great majority of drugs in each class. Of course, such a simple categorization in only four classes for all drugs cannot be expected to be universally true with no exceptions, as stated by Wu and Benet (5). It is even more difficult to hypothesize universal scientific explanations for these findings. However, those proposed by Wu and Benet (5) as possible explanations are as follows: Class 1 drugs are highly permeable and highly soluble, allowing them to pass through the gut lumen unaided by transporters. This will occur even if a Class 1 compound is a substrate *in vitro* for an uptake or efflux transporter expressed in the gut. This is hypothesized to occur because the gut is sufficiently leaky, so that the high permeability of these drugs does not give the transporter sufficient access to the drug or, alternatively, the drug's physicochemical properties allow it to achieve a high enough concentration in the lumen and within enterocytes that it will saturate any such transporter and be independent of transporter effects (5). Class 2 drugs are also highly permeable, so they will generally be able to enter enterocytes by passive diffusion, unaided by uptake transporters. However, due to low solubility limiting lumenal concentration, they are unlikely to saturate efflux transporters. Consequently, Class 2 compounds can be effluxed out of enterocytes and subject to efflux transporter effects that can influence bioavailability and absorption rate.

Of the two explanations above for high permeability, only the first, i.e., that the hepatocyte is sufficiently leaky, so that the high permeability of these drugs does not give the transporter sufficient access to the drug, seems reasonable for Class 1 drugs. At this time, we have no good explanation for

High Solubility | Low Solubility

Class 1

High Solubility
Extensive Metabolism

Class 2

Low Solubility
Extensive Metabolism

Class 3

High Solubility
Poor Metabolism

Class 4

Low Solubility
Poor Metabolism

(Extensive Metabolism / Poor Metabolism on left axis)

Fig. 4. Biopharmaceutics Drug Disposition Classification System (BDDCS) as put forth by Wu and Benet (5).

Table IV. Classification of 172 Drugs by BDDCS adapted from Wu and Benet (5)

Class 1: High Solubility/Extensive Metabolism		Class 2: Low Solubility/Extensive Metabolism	
Abacavir	Ketoprofen	Albendazole	Ketoconazole
Acebutolol	Ketorolac	Amiodarone	Lansoprazole
Acetaminophen	Labetolol	Atorvastatin	Lopinavir
Albuterol	Levamisole	Azathioprine	Lovastatin
Allopurinol	Levodopa	Azithromycin	Mebendazole
Amitryptiline	Lidocaine	Carbamazepine	Mefloquin
Antipyrine	Meperidine	Carvediol	Nalidixic acid
Buspirone	Metoprolol	Chlorpromazine	Naproxen
Caffeine	Metronidazole	Cisapride	Nelfinavir
Chloramphenicol	Midazolam	Clofazamine	Nevirapine
Chlorpheniramine	Minocycline	Cyclosporine	Nifedipine
Codeine	Misoprostol	Danazol	Oxaprozin
Colchicine	Morphine	Dapsone	Phenytoin
Cyclophosphamide	Phenobarbital	Diclofenac	Piroxicam
Desipramine	Phenylalanine	Diflunisol	Praziquantel
Dexamethasone	Prednisolone	Efavirenz	Raloxifene
Diazepam	Primaquine	Felodipine	Rifampin
Diltiazem	Promazine	Flurbiprofen	Ritonavir
Diphenhydramine	Promethazine	Glipizide	Saquinavir
Disopyramide	Propranolol	Glyburide	Sirolimus
Doxepin	Pyrazinamide	Griseofulvin	Spironolactone
Enalapril	Quinidine	Haloperidol	Sulfamethoxazole
Ergonovine	Quinine	Ibuprofen	Tacrolimus
Ergotamine	Rosiglitizone	Indinavir	Tamoxifen
Ethinyl estradiol	Salicyclic acid	Indomethacin	Terfenadine
Fluoxetine	Sotolol	Itraconazole	Warfarin
Glucose	Theophylline	Ivermectin	
Hydralazine	Valproic acid		
Imipramine	Verapamil		
Isoniazid	Zidovudine		
Isosorbid dinitrate			

Class 3: High Solubility/Poor Metabolism		Class 4: Low Solubility/Poor Metabolism	
Acyclovir	Ganciclovir	Acetazolamide	
Amiloride	Hydrochlorothiazide	Aluminum hydroxide	
Amoxicillin	Lamivudine	Amphotericin	
Atenolol	Levofloxacin	Chlorothiazide	
Atropine	Lisinopril	Chlorthalidone	
Bidisomide	Lithium	Ciprofloxacin	
Bisphosphonates	Lomefloxacin	Colistin	
Captopril	Metformin	Digoxin	
Cefazolin	Methotrexate	Furosemide	
Cetirizine	Metoclopramide	Neomycin	
Chloroquine	Nadolol	Nystatin	
Cimetidine	Neostigmine	Ofloxacin	
Cloxacillin	Penicillins	Phenazopyridine	
Dicloxacillin	Pitavastatin	Talinolol	
Doxycycline	Pravastatin		
Ephedrine	Pyridostigmine		
Erythromycin	Ranitidine		
Ethambutol	Riboflavin		
Famotidine	Tetracycline		
Fexofenadine	Trimethoprim		
Fluconazole	Valsartan		
Folinic acid	Zalcitabine		

why certain (but not all) Class 2 drugs are substrates for hepatic uptake and efflux transporters. Perhaps the explanation of Fagerholm (42) that difference in the degrees of "high" permeability dictate whether the compound will be a substrate for transporters is correct, but this requires further investigation. Class 3 compounds have high solubility but low permeability, while Class 4 compounds have both low solubility and low permeability. For both of these classes, drug is unable to get into enterocytes unless aided by an uptake transporter. Even for highly soluble Class 3 drugs, the need for uptake transport will limit the intra-enterocyte drug concentration, making saturation of efflux transporters un-

	High solubility	Low solubility
Extensive Metabolism	**Class 1** Transporter effects minimal	**Class 2** Efflux transporter effects predominate in the gut, while absorptive and efflux transporter effects occur the in liver
Poor Metabolism	**Class 3** Absorptive transporter effects predominate (but may be modulated by efflux transporters)	**Class 4** Absorptive and efflux transporter effects could be important

Fig. 5. Transporter effects predicted by BDDCS following oral dosing.

likely. Therefore, both Class 3 and Class 4 drugs may be subject to uptake and efflux transporter effects.

ORAL BIOAVAILABILITY (F) AND EXPOSURE (AREA UNDER THE CONCENTRATION-TIME CURVE)

Oral bioavailability is defined as the fraction of a dose administered that is absorbed into systemic circulation intact (43). It is a function of the fraction of the dose that is absorbed in the enterocytes (F_A), the fraction of the absorbed dose that passes through the gut membranes intact (F_G), and the fraction passing through the liver intact (F_H) before reaching the systemic blood supply (Eq. 1). Transporters will help modulate F of drugs by playing a role in how much drug gets across the gut membranes and how much drug is taken up into hepatocytes.

$$F = F_A \times F_G \times F_H \quad (1)$$

Bioavailability is proportional to the area under the concentration-time curve (AUC), which is a measure of drug exposure (Eq. 2). Consequently, transporter effects on F will result in a corresponding effect on AUC. Changes in AUC are easier to determine than those on F, since both *in vivo* oral and IV studies are needed to quantitate absolute bioavailability. Many drugs are only available in either oral or IV formulations, but not both.

$$F = (CL \cdot AUC)/DOSE \quad (2)$$

The concept of bioavailability relates to Eq. 2. For an IV dose, bioavailability is assumed to be complete, i.e. $F=1$. Thus, assuming clearance (CL) to be constant between the IV and oral dosings, oral F may be determined by comparing exposure for oral and IV doses, while correcting for any differences in dose between the two studies. *In situ* and *ex situ* experiments with individual organs, such as the intestine or liver, using perfusion techniques are very useful for studying transporter effects on F, and coupling results from these organ experiments with whole animal and human AUC data can help elucidate the mechanisms involved in altering both bioavailability and drug exposure.

In the gut, Class 1 and 2 compounds have favorable permeability characteristics, allowing them to enter enterocytes unaided by apical uptake transporters. As explained above, due to their high solubility and rapid permeability, Class 1 compounds also readily bypass efflux transporters or, alternatively, the high concentrations likely saturate apical efflux transporters, and, therefore, Class 1 drug AUC and F will not be influenced by efflux transporters even if they are substrates *in vitro*. Cao *et al*. (44) demonstrated this *in situ* with an intestinal rat perfusion model in which they determined verapamil and propranolol permeabilities and showed that they were independent of Pgp expression level, which increased 6-fold along the intestinal tract from the duodenum to the ileum. While the status of propranolol as a Pgp substrate is unclear, it is well recognized that verapamil is a good Pgp substrate. Although AUC and F of Class 1 compounds are independent of apical efflux transporters, such efflux transporters will influence AUC and F of Class 2 compounds. Due to their low solubility, the low concentration of Class 2 compounds should not saturate apical efflux transporters as Class 1 compounds can. Therefore, apical efflux transporters will be able to pump drug back out into the lumen where it may re-enter enterocytes, thus allowing repeated exposure to drug metabolizing enzymes within the enterocytes. This interplay between apical gut efflux transporters and metabolizing enzymes within enterocytes has been well-characterized using *in vitro* and *in vivo* systems (45,46). Cummins *et al*. used both a Caco2 cell line overexpressing CYP3A4 (45) and a rat intestinal perfusion model (46) to demonstrate the importance of Pgp in influencing metabolism of K77, a cysteine protease inhibitor and dual substrate of Pgp and CYP3A4. In brief, both the cell line studies and the intestinal perfusion studies showed that Pgp inhibition increased intracellular K77 concentrations or appearance in mesenteric blood while decreasing polarized transport. No corresponding changes were seen for the CYP3A4 substrates felodipine (45), a Class 2 compound that is not a substrate of Pgp, or midazolam (46), a Class 1 compound not influenced by Pgp in the gut. The effects of inducing Pgp have been shown to yield opposite effects to that for Pgp inhibition *in vivo* with the Class 2 (BCS)/Class 4 (BDDCS) compound talinolol. Schwarz *et al*. (47) induced gut Pgp by pretreatment with St. John's Wort and saw a significant reduction (25%) in F compared to the water control. Likewise, Westphal *et al*. (48) saw a significant (~20%) reduction in F for talinolol when Pgp was induced with rifampin. While the interplay between Pgp and CYP3A4 has been extensively studied, this phenomenon may also occur with other enterocytic drug metabolizing enzymes (CYP3A4, CYP2C9, CYP2C19, CYP2C8, CYP2D6, esterases, epoxide hydrolases, UGT1A1, UGT1A7–10, SULT1E1, SULT2A1, SULT1A3, N-acetyltransferases, glutathione-S-transferases) (49), and other apical efflux transporters (i.e., BCRP, MRP2). One such example is provided by Sesink *et al*. (50). Using Mrp2 knockout mice and the specific Bcrp1 inhibitor FTC, they showed in an *in situ* intestinal perfusion model that inhibiting apical efflux of quercetin glucuronides by Bcrp1 increased plasma quercetin levels

more than 2-fold after enzymatic hydrolysis of the plasma samples. Another example is provided by Su *et al*. (51) in which rat ileal perfusions were performed with topotecan in the presence and absence of novobiocin, a Bcrp1 inhibitor. With novobiocin, a ~50% decrease in intestinal secretion was seen with a corresponding increase in permeability, indicating that Bcrp1 inhibition influences topotecan absorption and bioavailability. Currently it is difficult to study BCRP and MRP2 interactions in human clinical studies due to the lack of specific inhibitors that can be used in people. Also, many drugs are substrates for multiple efflux transporters. Pgp and BCRP have extensive substrate overlap, and, therefore, one transporter may compensate when the other is inhibited. In preclinical studies, the use of knockout animals can circumvent this problem, but an effective strategy for human studies remains to be found. More studies with these other gut enzymes and efflux transporters are needed to fully characterize the importance of their potential interplay in influencing AUC and F of substrate drugs.

BDDCS Class 3 and 4 drugs, which have low permeability, require apical uptake transporters to help them cross the luminal barrier, since their low permeability limits diffusion into enterocytes. Clinical studies have shown that inhibition of apical gut uptake transporters can also influence plasma levels of substrate drugs. Glaeser *et al*. (52) showed that administration of grapefruit juice concomitantly or 2 h prior to dosing the Class 3 compound fexofenadine, an OATP1A2 substrate, decreased fexofenadine plasma levels compared to water control, as well as dosing of grapefruit juice 4 h prior to fexofenadine dosing. Since fexofenadine, like all BDDCS Class 3 drugs, is not extensively metabolized, the authors concluded that the effect was due to inhibition of OATP1A2 uptake of fexofenadine. Kato *et al*. (53) demonstrated in mouse pharmacokinetic and Ussing chamber studies that celiprolol absorption was significantly decreased by bromosulfophthalein (BSP), a broad organic anion transport inhibitor. They used mdr1a/1b knockout mice to avoid any confounding effects of Pgp and demonstrated decreased plasma levels of celiprolol upon BSP coadministration in the pharmacokinetic studies. They confirmed that the effect was due to decreased absorption in Ussing chamber studies by showing that BSP significantly reduced A to B permeability. Induction of intestinal uptake transporters conversely should increase the AUC and F of their substrates. A rat pharmacokinetic study by Koitabashi *et al*. (54) demonstrated that administration of orange juice significantly increased the AUC of orally dosed pravastatin while having no effect on IV pravastatin, indicating a change in F. They further investigated mRNA and protein levels of rat Oatp1a1 and Oatp1a4 and saw significant increases in both for each transporter. In addition to the rat studies, Koitabashi *et al*. (54) also ran a human study to examine the effect of orange juice on oral pravastatin. As with the rats, a significant increase in AUC was seen, although a corresponding IV study was not possible to provide evidence that the change was due to increased F.

Class 3 and 4 drugs also have the potential to be influenced by efflux transporters, since they are unlikely to attain intracellular concentrations high enough to saturate efflux transporters. Dahan *et al*. (55) examined the effect of intestinal Pgp on two Class 3 compounds, famotidine and

cimetidine. They determined in rat *in situ* intestinal perfusion studies that differential Pgp expression in the proximal jejunum *versus* the distal ileum led to different famotidine and cimetidine permeabilities in these segments with significantly decreased permeability seen in the distal segment compared to the proximal segment, corresponding to the higher Pgp expression observed in the distal segment. Upon Pgp inhibition by verapamil, a significant increase in permeability for both compounds was seen in the distal ileum segment, while no significant difference was seen in the proximal jejunum. The distal ileum permeability approached the permeability seen in the proximal jejunum segment, effectively making permeability constant along the small intestine in the presence of verapamil. Evidence supporting the effect of apical gut efflux on BDDCS Class 4 drug absorption also exists for digoxin, a Pgp substrate. A human clinical study by Greiner *et al*. (56) showed that induction of Pgp by rifampin decreased the absorption of orally dosed digoxin, even though digoxin is not metabolized by CYPs in humans. Subjects received either IV or oral digoxin before and after Pgp induction. Pgp induction was examined by immunohistochemistry and Western blot analysis of duodenum samples and was shown to increase 3.5-fold after ~2 weeks of rifampin dosing. F was significantly decreased (19%) after Pgp induction. Further work is required to fully characterize the effect of gut apical transporters on Class 3 and 4 drugs.

The influence of intestinal basolateral uptake and efflux transporters has not yet been examined in depth to determine their influence on AUC and F; however, we can predict what effects they may have based on what we predict for the effects of apical transporters. Table V summarizes the effects of intestinal drug transporters on AUC by BDDCS class.

The BDDCS is also useful for making predictions regarding food effects on orally dosed drugs. The AUC and F of many compounds are greatly affected by concomitant food intake, and the FDA recommends that high fat meals (800–1000 cal: 50–65% from fat, 25–30% from carbohydrates, 15–20% from protein) be used in food-effect studies in the guidance "Food Effect Bioavailability and Fed Bioequivalence Studies" (57). Many factors are believed to contribute to these food effects, including changes in gastric emptying time, bile flow, pH of the intestine, splanchnic blood flow, and gut wall metabolism. A variety of evidence exists supporting food effects on transporters as well and is described in detail in a review by Custodio *et al*. (58). The BDDCS is able to

Table V. Predicted Effect of Intestinal Drug Transporters on Exposure (AUC) by BDDCS Class

BDDCS Class	1	2	3	4
Inhibition				
Apical Uptake	⟺	⟺	⇓	⇓
Apical Efflux	⟺	⇑	⇑	⇑
Basolateral Uptake	⟺	?	⇑	⇑
Basolateral Efflux	⟺	?	⇓	⇓
Induction				
Apical Uptake	⟺	⟺	⇑	⇑
Apical Efflux	⟺	⇓	⇓	⇓
Basolateral Uptake	⟺	?	⇓	⇓
Basolateral Efflux	⟺	?	⇑	⇑

predict what is seen clinically in food effect studies. These predictions are explained if it holds that high-fat meal components inhibit apical gut uptake and efflux transporters. Fig. 6 summarizes the effects of high fat meals on F and t_{max} by BDDCS Class (5,58).

Drug that makes it across the intestinal barrier passes into the hepatic portal vein and travels to the liver, the final major site for first-pass effects, before reaching the systemic circulation. Drug transporters will play a role in determining how much drug enters hepatocytes and is exposed to hepatic drug metabolizing enzymes and biliary excretion. In hepatocytes, basolateral uptake transporters function to take drug up from the sinusoidal blood into hepatocytes while basolateral efflux transporters pump drug back out into the sinusoidal blood. Apical transporters face the bile ducts; therefore, apical efflux transporters will pump drug into the bile.

The major predictive difference for the BDDCS system between the gut and the liver relates to the importance of uptake transporters for BDDCS Class 2 drugs. It is possible that the intestinal membrane may be "leakier" than the hepatic membrane, allowing more drug to diffuse across the intestinal barrier than the hepatic barrier. For example, while BDDCS Class 2 compounds do not require uptake transporters to get into enterocytes, it has been shown that hepatic uptake transporters can be an important determinant of drug entry into hepatocytes and could thereby affect F through the first pass effect and AUC through the combination of the first pass effect and subsequent passages through the liver. Tirona *et al.* (59) demonstrated that the Class 2 compound rifampin could be transported by OATP1B1 and, to a lesser extent, OATP1B3 expressed in HeLa cells. Su *et al.* (60) determined that OATP1A2 is able to transport saquinavir, also a Class 2 compound, in oocytes and in HepG2 cells, a human hepatocellular liver carcinoma cell line. The AUC and F of Class 3 and 4 drugs, due to their low permeability, will also be influenced by hepatic uptake transporters. Watanabe *et al.* (61) investigated the importance of hepatic uptake trans-

Table VI. Predicted Hepatic Effect of Drug Transporters on Exposure (AUC) by BDDCS Class

BDDCS Class	1	2	3	4
Inhibition				
Apical Efflux	⟺	⇩	⇧	⇧
Basolateral Uptake	⟺	⇧	⇧	⇧
Basolateral Efflux	⟺	⇩	⇩	⇩
Induction				
Apical Efflux	⟺	⇧	⇩	⇩
Basolateral Uptake	⟺	⇩	⇩	⇩
Basolateral Efflux	⟺	⇧	⇧	⇧

porters on the plasma levels of the Class 3 compound pravastatin. They developed a physiologic based pharmacokinetic (PBPK) model that incorporated hepatic sinusoidal uptake and demonstrated the sensitivity of plasma levels to a change in uptake. Decreasing uptake transporter activity to 1/3 of control led to simulated pravastatin plasma levels 271% of the control, while increasing uptake transporter activity to 3x the control led to simulated plasma levels 14% of control. Hepatic basolateral efflux transporters (i.e., MRP3, 4, 5, 6) may influence AUC and F since they provide an avenue of exit from hepatocytes. As in the intestine, it is possible that these membrane-bound transporters can interact with drug before it interacts with metabolizing enzymes associated with the endoplasmic reticulum. Altering these transporters' function could affect the AUC and F of their substrates by altering first pass hepatic metabolism or biliary excretion. Theoretically, the apical efflux transporters could also affect AUC and F by helping to control intra-hepatocyte drug levels, although this effect would be dependent on the drug's ability to get back out of the hepatocyte into sinusoidal blood either by diffusion or by basolateral efflux. A summary of the effects of hepatic drug transporters on AUC is given in Table VI and explicated in the next section.

CLEARANCE (CL)

Clearance is technically defined as a proportionality factor that relates the rate of elimination of drug to the systemic drug concentration (43). It must be kept in mind that metabolism that occurs in the gut and first pass in the liver does not contribute to CL, since that drug is lost before it reaches systemic circulation. For orally dosed drugs, CL and F are intimately associated and cannot be separated unless corresponding IV studies have been done. Since this review deals with orally dosed drugs, we will discuss transporter effects on CL/F in addition to the intrinsic clearance by the liver.

$$CL = F \cdot DOSE/AUC \qquad (2a)$$

$$CL/F = DOSE/AUC \qquad (2b)$$

Gut transporters will exclusively affect F, thus CL will not change in response to induction or inhibition of gut transporters, only F will. For orally dosed drugs the liver and
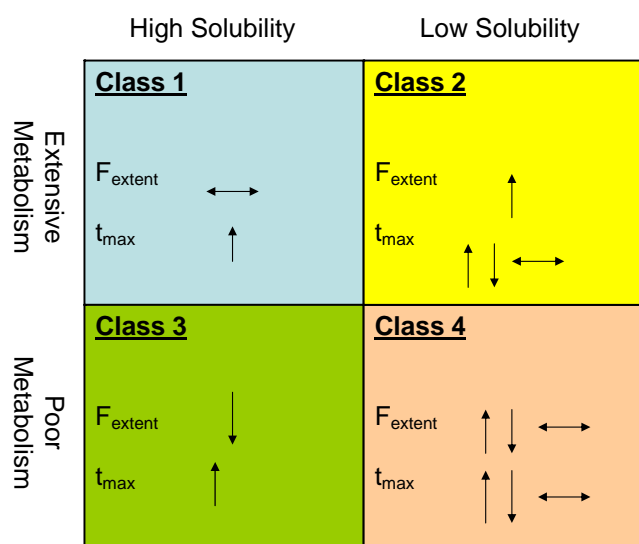

Fig. 6. Predicted high fat meal effects by BDDCS classification after Wu and Benet (5).

the kidneys are the major eliminating organs, and their clearances are additive.

$$\text{Total CL} = CL_H + CL_R \qquad (3)$$

CL_H can further be divided into uptake and elimination processes, with the latter divided into biliary excretory clearance and hepatic metabolic clearance (43). Both mechanisms can be affected by transporters.

Transporters in the liver and kidney can affect the CL of the portion of an orally administered drug that escapes the first-pass effect. Hepatic uptake transporters are expressed on the basolateral surface of hepatocytes facing the sinusoidal blood supply. Inhibiting these transporters prevents drug from being exposed to metabolizing enzymes within hepatocytes, decreasing the CL_H of drugs that are substrates for these enzymes. For drugs that are not metabolized but are eliminated by biliary excretion, CL_H may also decrease, since drug is prevented from getting into the bile through the hepatocytes. Conversely, induction of hepatic uptake transporters will increase the amount of drug able to get into hepatocytes and thus gain exposure to metabolizing enzymes or get pumped into the bile, thereby increasing CL_H. CL/F will change in the same direction as CL_H for each case, but the CL value is not separable from F based on oral data alone. In addition to basolateral uptake transporters, hepatocytes also express basolateral efflux transporters that pump drug back out into the sinusoidal blood. The effects of inhibiting or inducing these transporters will be opposite those for the basolateral uptake transporters. Inhibiting basolateral efflux will cause more drug to remain inside hepatocytes and will lead to increased CL_H either by increased exposure to metabolizing enzymes or to efflux into the bile, while inducing them will lead to more drug being pumped out of hepatocytes and decreased CL_H. Again, CL/F will change in the same direction as CL_H.

Class 1 compounds, due to their high permeability, appear not to be influenced by basolateral hepatic uptake transporters. In the gut, Class 2 compounds are not strongly influenced by apical uptake transporters due to their high permeability; however, it has been shown that at least some of these compounds are influenced by basolateral hepatic uptake transporters. A human pharmacokinetic study by Lau *et al.* (62) showed that an IV dose of the OATP inhibitor rifampin significantly decreased CL/F of atorvastatin, presumably by decreasing basolateral uptake by OATP1B1 leading to decreased hepatic metabolism by CYP3A4. *In vitro* studies showed that 50 μM rifampin did not inhibit CYP3A4 metabolism of atorvastatin, and rifampin plasma levels in the human study were ~15 μM, indicating that inhibition of hepatic metabolism was not a causal factor in reducing CL/F. Similar results were seen by Zheng *et al.* (63) in a human study examining the effect of IV rifampin on orally dosed glyburide, an OATP2B1 substrate. Upon multiple dosing of rifampin, but when rifampin was no longer present in the systemic circulation, glyburide CL/F was significantly increased compared to the control, due to induction of metabolic enzymes and transporters. As stated earlier, we have no good explanation for why some BDDCS class 2 compounds are substrates for hepatic uptake transporters but this is not seen for BDDCS class 1 compounds. It is difficult to imagine a scientific explanation based on a

solubility effect in the liver. This is an area requiring further investigation.

As expected due to their low permeability potential, the CL/F of Class 3 and 4 drugs also can be influenced by basolateral hepatic uptake transporter effects. Studies with the Class 3 drugs pravastatin and pitavastatin have shown the effects of decreased function of OATP1B1 on CL/F. Deng *et al.* (64) performed human clinical studies looking at the effect of genotype of OATP1B1 (OATP1B1*1a homozygotes *versus* OATP1B1*15 homozygotes) on pitavastatin and pravastatin pharmacokinetics. They saw that CL/F for both compounds was significantly decreased in subjects with OATP1B1*15/*15 genotype compared to wildtype (2.07-fold and 2.88-fold for pravastatin and pitavastatin, respectively). Metformin, another Class 3 compound and a substrate for OCT1, has also shown transporter influence on pharmacokinetic parameters. Shu *et al.* (65) performed a clinical study to determine the effect of OCT1 genetic variation on metformin pharmacokinetics. Metformin was orally dosed in two subject groups, one group with wildtype OCT1 and the other composed of subjects carrying at least one reduced function allele. They found that the CL/F was significantly reduced in the variant allele group.

Hepatic apical efflux transporters responsible for pumping drug into the bile may also affect both hepatic metabolic clearance and biliary excretory clearance of their substrate drugs. Inhibiting apical efflux causes drug to remain trapped within hepatocytes. If the compound is a substrate for metabolizing enzymes (BDDCS Class 1 and 2 compounds), its metabolism will increase due to increased exposure to enzymes, and CL_H will increase. If the drug is not subject to metabolic transformation but rather is eliminated by biliary excretion (Class 3 and 4 compounds), its CL_H may decrease due to decreased excretion into bile. An alternative route of elimination is possible though, which may cause a situation to arise in which there is no net change in CL/F, but rather a change in the clearance profile seen as decreased biliary excretion and increased renal excretion if the drug is sufficiently hydrophilic to be eliminated in the urine. Induction of hepatic apical efflux transport could lead to increased biliary excretion but decreased metabolic transformation, making the effect on CL_H dependent upon a drug's substrate status for metabolizing enzymes. Work remains to be done to elucidate the effect of hepatic apical efflux transporters on CL/F separate from that of apical gut efflux transporters.

While work has been done on determining effects of hepatic basolateral efflux in *in situ* isolated perfused rat liver studies (66) and *in vivo* with intraperitoneal dosing (67), the effect of hepatic basolateral efflux transporters on oral drug pharmacokinetics has not been extensively studied. However, we can hypothesize what effects inhibition or induction will have. Inhibition of hepatic basolateral efflux will cause drug to be trapped within hepatocytes, similar to hepatic apical efflux inhibition, and will lead to increased exposure to drug metabolizing enzymes or to apical efflux. This may lead to an increase in hepatic metabolic or biliary excretory clearance. Induction of hepatic basolateral efflux will cause more drug to be pumped back into the sinusoidal blood, decreasing the hepatic contribution to CL/F, but depending on the drug's hydrophilicity may be compensated for by increased renal CL.

HALF-LIFE (T$_{1/2}$)

The elemination half-life is dependent upon CL and V (Eq. 4).

$$t_{1/2} \approx 0.693 \times V/CL \qquad (4)$$

Whatever effects transporters exert on V and CL will influence the $t_{1/2}$, either causing it to increase if the increase in V predominates, decrease if the increase in CL is more pronounced, or have no net effect if changes in V and CL cancel out each other. However, transporters in the gut may affect the absorption half-life, $t_{1/2,abs}$ (Eq. 5).

$$t_{1/2,abs} = .693/k_a \qquad (5)$$

The rate constant of absorption, k_a, may be influenced by gut transporters, and the BDDCS can be useful in predicting which drugs are likely to be affected. As stated before, Class 3 and 4 drugs are dependent on gut uptake transporters to get across the luminal membrane. If their uptake is inhibited, this is likely to decrease their absorption rate, driving down k_a and increasing $t_{1/2,abs}$. The opposite will hold true when uptake transporters are induced. The k_a will increase and $t_{1/2,abs}$ will decrease as more drug is pumped into enterocytes. Parker et al. (68) ran a human study in which a single oral dose of digoxin was administered after 5 days pretreatment with grapefruit juice and saw a significant decrease in the digoxin k_a compared to the water control arm (3.0 ± 2.4 to 1.2 ± 1.0 hr^{-1}, $p < 0.05$). The plasma concentration vs. time profile also showed a later t_{max}. No other pharmacokinetic parameters were significantly different between the treatment groups. The k_a and t_{max} results were not what would be expected if the interaction were due to Pgp inhibition (68,69), but rather what would be expected from inhibition of an intestinal uptake transporter. Although the specific OATP isoform responsible for intestinal uptake of digoxin has not been identified, the drug is known to be an OATP1B3 (70) substrate. The BDDCS predicts the clinical results seen by Parker et al., since digoxin is a Class 4 compound in humans and uptake transporter effects will be important for these compounds. Intestinal efflux transporters may also affect k_a and $t_{1/2,abs}$, especially for Class 2 compounds. If apical efflux transporters are inhibited, this will decrease the "cycling" effect that is seen when a drug is repeatedly pumped out and gains reentry into enterocytes, allowing the drug to exit basolaterally more quickly. In a study on the effects of ABCB1 genotype, Solas et al. (71) found that subjects dosed with indinavir (Class 2 compound) carrying one decreased-function allele, C3435CT, had a significantly higher k_a (2-fold) compared to controls and carriers of two mutant alleles (they explain the lack of significant difference in the C3435TT group as possibly due to lack of statistical power with the small number of subjects). The $t_{1/2,abs}$ of Class 3 and 4 compounds may also be subject to apical efflux transporter effects. For the Class 3 compound fexofenadine, a Pgp substrate (72,73), Tannergren et al. (74) determined in human intestinal perfusion studies with the Gut-I-Loc system that coperfusion of fexofenadine with the Pgp inhibitor verapamil caused a significant increase in k_a compared to the control (0.0030 ± 0.0012 min^{-1} to $0.0255 \pm$

0.0103 min^{-1} ($P < .001$)), which changes $t_{1/2,abs}$. Decreasing the absorption rate often changes the appearance of the plasma concentration vs. time profile, even if no change in the extent of absorption as measured by area under the curve (AUC) is seen. The t_{max} can shift later and C_{max} can decrease, causing the profile to appear flatter. Increasing the absorption rate constant may cause t_{max} to shift earlier and C_{max} to increase, sharpening the plasma concentration vs. time profile. Class 1 drugs, for which transporter effects are minimal, should not show a significant change in the absorption rate constant or $t_{1/2,abs}$.

Transporter effects on k_a may also have implications for oral multiple dosing regimens. In a recent analysis, Sahin and Benet (75) defined a new half-life parameter, the operational multiple dosing half-life ($t_{1/2,op}$), as "the half-life equal to the dosing interval at steady-state where the maximum concentration at steady-state is twice the maximum concentration found for the first dose and where the fall off to the trough concentration from the maximum concentration is consistent with this half-life." They found that this $t_{1/2,op}$ parameter for some drugs may be quite sensitive to changes in k_a. In simulations with diazepam, decreasing the k_a from 2.77 h^{-1} to 0.347 h^{-1} led to a drastic increase in the $t_{1/2,op}$, increasing it from about 13 h to 35 h. Diazepam is a Class 1 drug; therefore, we would not expect to see transporter effects on k_a. However, further study is warranted to determine what effect varying k_a has on the $t_{1/2,op}$ for Class 2, 3, and 4 compounds through transporter interactions.

VOLUME OF DISTRIBUTION (V)

Volume of distribution is defined as the "volume into which a drug distributes in the body at equilibrium (43)" and is a "direct measure of the extent of distribution (43)." Transporters in the gut will not affect V as it relates the concentration of drug in the systemic circulation to amount of drug in the body. Like CL, V for orally administered drugs cannot be separated from F, and V is reported as V/F. Very few drugs remain within physiologically realistic volumes (43), such as the plasma (3 L), extracellular water (16 L), or total body water (42 L). Instead they distribute into organs and tissues, greatly increasing their apparent V. Transporters may influence V by mediating their transport into and out of a variety of tissues and organs. The most widely studied sites thus far in relation to transporter influence on distribution are the liver and the brain, but the principles determined for these organs should hold for other organs and tissues expressing drug transporters. Several drugs whose site of action is the liver, including some statins, are concentrated in the liver by hepatic uptake transporters. If their hepatic uptake is inhibited, more drug remains in the plasma and less drug gets into the liver, decreasing V/F. For example, Lau et al. (62) saw a significant reduction in V_{ss}/F in response to a single IV dose of rifampin inhibiting the hepatic uptake of atorvastatin. Zheng et al. (63) showed a similar effect of rifampin on the V_{ss}/F for glyburide. Induction may increase the amount of drug getting into the liver, decrease the amount in plasma, and increase V/F. While the effects of altering function of hepatic apical efflux transporters may influence V/F, this effect isn't always predictable. Decreasing hepatic basolateral efflux may keep drug within hepatocytes, possibly

decreasing plasma levels and increasing V/F, while inducing basolateral efflux may lead to decreased V/F by causing there to be more drug in the plasma.

An extensive review of transporter effects on V has recently been published (170).

CONCLUSIONS

A wealth of knowledge exists regarding drug transporters, and increasingly their effects on pharmacokinetics are being characterized. Work in the field is far from complete though, and there are many areas in which there is a dearth of information.

While the substrate and inhibition profiles of transporters both new and old are being updated continuously (76–78), new transporters are still being discovered. For example, Engel *et al.* (79) recently cloned the proton-stimulated organic cation transporter (PMAT) expressed in the intestine, and Zhou *et al.* (80) determined that it may play a role in intestinal transport of metformin. The search goes on for appropriate probe substrates (81–83) and specific inhibitors (84–86) that will allow testing of single transporters without confounding effects from substrate overlap (12,87,88).

With widespread use of cell models to test substrate profiles and to determine permeabilities, care needs to be taken to ensure that reproducible results are obtained and to be aware of factors that can lead to discrepancies between different laboratories' results (36,89,90).

The development of models that incorporate different transporters is a huge step forward in the field; however, these models need valid *in vivo* parameter values in order to accurately predict pharmacokinetics under a variety of conditions (32,91–95). Human and animal studies need to continue to generate data to develop complete and robust *in silico* models.

The understanding of transporter effects on oral pharmacokinetics has improved vastly over the last 20 years, yet it is still a vibrant and important area of research needing continued effort and advancement.

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