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Commentary

Predicting Drug Disposition via Application of BCS: Transport/Absorption/ Elimination Interplay and Development of a Biopharmaceutics Drug Disposition Classification System

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The Biopharmaceutics Classification System (BCS) was developed to allow prediction of *in vivo* pharmacokinetic performance of drug products from measurements of permeability (determined as the extent of oral absorption) and solubility. Here, we suggest that a modified version of such a classification system may be useful in predicting overall drug disposition, including routes of drug elimination and the effects of efflux and absorptive transporters on oral drug absorption; when transporter-enzyme interplay will yield clinically significant effects (e.g., low bioavailability and drug-drug interactions); the direction, mechanism, and importance of food effects; and transporter effects on postabsorption systemic drug concentrations following oral and intravenous dosing. These predictions are supported by a series of studies from our laboratory during the past few years investigating the effect of transporter inhibition and induction on drug metabolism. We conclude by suggesting that a Biopharmaceutics Drug Disposition Classification System (BDDCS) using elimination criteria may expand the number of Class 1 drugs eligible for a waiver of *in vivo* bioequivalence studies and provide predictability of drug disposition profiles for Classes 2, 3, and 4 compounds.

KEY WORDS: BCS; BDDCS; disposition; drug interactions; food effects; routes of elimination; transporter-enzyme interplay.

INTRODUCTION

Amidon and co-workers (1) recognized that the fundamental parameters controlling the rate and extent of oral drug absorption were the drug's aqueous solubility and gastrointestinal permeability. They devised a Biopharmaceutics Classification System (BCS) that categorized drugs into four classes according to their solubility and permeability (expressed as the extent of oral drug absorption) as depicted in Fig. 1. In 2000, the FDA used the BCS system as a science-based approach to allow waiver of *in vivo* bioavailability and bioequivalence testing of immediate-release solid dosage forms for Class 1 high-solubility, high-permeability drugs when such drug products also exhibit rapid dissolution (2).

At its core, the BCS is an experimental model, centrally embracing permeability and solubility, with qualifications related to pH and dissolution. The objective of the BCS is to predict *in vivo* pharmacokinetic performance of drug products from measurements of permeability and solubility. A drug substance is considered "highly soluble" when the highest dose strength is soluble in 250 ml or less of aqueous media over a pH range of 1–7.5 at 37°C. A drug substance is considered to be "highly permeable" when the extent of the absorption (parent drug plus metabolites) in humans is determined to be ≥90% of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose. In Table I, we have assembled a list of compounds in the four BCS classes, predominantly gathered from the literature (1,3–18) but judiciously edited. With respect to oral bioavailability, it is generally believed that the framework of the BCS could serve the needs of the earliest stages of discovery research. In this manuscript, we demonstrate that categorizing drugs into the four classes represented by BCS solubility and permeability criteria may provide significant new insights to the pharmaceutical scientific community. This classification system may be useful in predicting routes of elimination, effects of efflux and absorptive transporters on oral absorption, when transporter-enzyme interplay will yield clinically significant effects such as low bioavailability and drug-drug interactions, the direction and importance of food effects, and transporter effects on postabsorption systemic levels following oral and intravenous dosing. We propose that a modest revision of the BCS criteria may result in a classification system that yields predictability of *in vivo* disposition for all four classes, as well as increasing the number of Class 1 drugs eligible for bioequivalence study waivers.

As we were preparing this manuscript, the extensive evaluation of the WHO Essential Medicines List in terms of BCS classification based on measured solubility and permeability/absorption data was published (18). We have modified the manuscript to include many of the compounds evaluated

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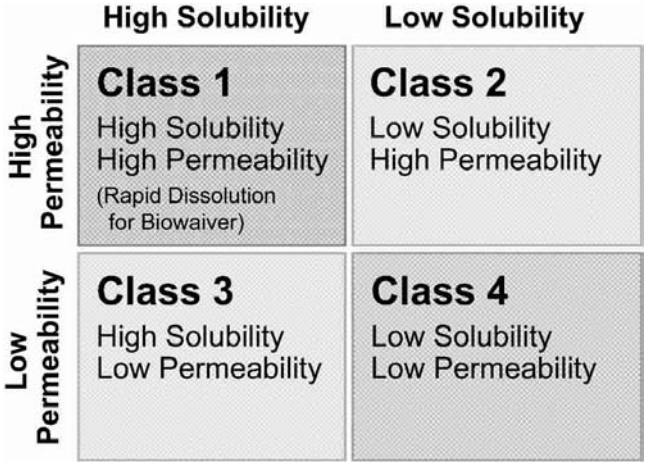

Fig. 1. The Biopharmaceutics Classification System (BCS) as defined by the FDA (2) after Amidon *et al.* (1).

in that work. We agree with most of the classifications assigned, but not all, as our paper expands the utility of the classification to drug disposition. We have added comments about some of these differences throughout the manuscript.

Predicting Routes of Drug Elimination

Examining the drug substances listed in the four BCS classes in Table I, it becomes obvious that Class 1 and Class 2 compounds are eliminated primarily via metabolism, whereas Class 3 and Class 4 compounds are primarily eliminated unchanged into the urine and bile (Fig. 2). We are unaware that this simple categorization under BCS has previously recognized the correlation and fact that the high permeability of the Classes 1 and 2 compounds allows ready access to the metabolizing enzymes within hepatocytes, although Smith (19) has noted that more permeable lipophilic compounds make good substrates for cytochrome P450 (CYP) enzymes. Note that the differential permeability characteristics defined under BCS do not necessarily reflect differences in permeability into hepatocytes, as a number of Class 3 and Class 4 compounds are eliminated into the bile. Rather, the high vs. low permeability designation reflects differences in access to the metabolizing enzymes within the hepatocytes.

For the 130 drugs/compounds listed in Table I, only 13 of the substances do not have readily accessible, critically evaluated pharmacokinetic parameters (20,21). Upon reviewing the disposition characteristics of the Class 3 and Class 4 drugs listed in Table I, all but mebendazole are eliminated predominantly in the unchanged form by the renal or biliary route. We suspect that mebendazole is misclassified, as it is extensively metabolized [note that Lindenberg *et al.* (18) most recently listed mebendazole as either Class 2 or Class 4]. We propose that for the purposes of defining the BCS classification for predicting drug disposition, the extent of metabolism may be a better predictor than the 90% absorption characteristic.

One might suspect that the high-permeability compounds (Class 1 and Class 2) should have higher volumes of distribution than the low-permeability Class 3 and Class 4 compounds. When evaluating the published pharmacokinetic characteristics (20,21), we observed such a trend, but the concordance is not even close to that found between BCS class and major routes of elimination. Many highly protein bound acidic Class 1 and Class 2 compounds exhibit very low volumes of distribution (e.g., valproic acid, ibuprofen). It would be incorrect, however, to conclude that correction for protein binding would give a better prediction of the relative size of the volume of distribution in comparing Classes 1 and 2 compounds with Classes 3 and 4 drugs. In fact, our analysis demonstrates that the generally larger volumes of distribution for Class 1 and Class 2 compounds when compared to Class 3 and Class 4 compounds is independent of the degree of protein binding.

Most New Molecular Entities Are Class 2 Compounds

New molecular entities (NMEs) today are frequently large-molecular-weight, lipophilic, poorly water-soluble compounds that most often fall into BCS Class 2. Lipinski *et al.* (22) pointed out that leads obtained through high-throughput screening (HTS) tend to have higher molecular weights and greater lipophilicity than leads in the pre-HTS era. Lipinski's Rule of 5 was developed to set "drugability" guidelines for NMEs (23). In the drug discovery setting, the Rule of 5 predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500, and the calculated Log P (CLog P) is greater than 5. However, Lipinski specifically states that the Rule of 5 only holds for compounds that are *not* substrates for active transporters (22,23). When the Rule of 5 was developed, information about drug transporters was very limited. We believe that almost all drugs are substrates for some transporter. Studies to date have not been able to show this because we are just beginning to gain the knowledge and tools that allow investigation of substrates for uptake transporters. In addition, unless a drug molecule can passively gain intracellular access, it is not possible to simply investigate whether the molecule is a substrate for efflux transporters.

Lipinski has noted that the Rule of 5 was intended as a very crude filter (24). Thus, it is not surprising that predictions based only on solubility and Log P or CLog P may frequently be in error, often because most drugs may be substrates for some transporter. We note that a recent evaluation of the provisional biopharmaceutical classification of WHO essential drugs (25) reported a generally good correlation between *in silico* parameters and BCS classification; however, some obvious misclassifications occurred. For example, acetaminophen (bioavailability = 88%), dapsone (93%), and theophylline (96%), all highly metabolized drugs, are listed as Class 4 compounds based only on physicochemical criteria (25), as opposed to their Classes 1 and 2 listings in Table I.

Cautions

Prior to making further predictions related to transporter-enzyme interactions, food effects and drug-drug interactions, we wish to provide the following cautions.

a) There will always be exceptions to the broad general rules presented here (e.g., the Class 2 compound digoxin does not undergo extensive hepatic metabolism in humans, but it does in the rat). As research scientists, we find exceptions to predictability (and unexpected events) more intriguing and challenging than the expected or predictable events. As in

Table I. Biopharmaceutics Classification System (BCS) Substrates[a]

	High solubility		Low solubility	
High permeability	**Class 1**		**Class 2**	
	Abacavir	**Ketorolac**	**Amiodarone**[I]	**Itraconazole**[S,I]
	Acetaminophen	Ketoprofen	**Atorvastatin**[S,I]	**Ketoconazole**[I]
	Acyclovir[b]	Labetolol	**Azithromycin**[S,I]	**Lansoprazole**[I]
	Amiloride[S,I]	Levodopa[S]	**Carbamazepine**[S,I]	**Lovastatin**[S,I]
	Amitryptyline[S,I]	Levofloxacin[S]	**Carvedilol**	*Mebendazole*
	Antipyrine	**Lidocaine**[I]	Chlorpromazine[I]	Naproxen
	Atropine	Lomefloxacin	**Cisapride**[S]	Nelfinavir[S,I]
	Buspirone[c]	**Meperidine**	*Ciprofloxacin*[S]	Ofloxacin
	Caffeine	Metoprolol	**Cyclosporine**[S,I]	Oxaprozin
	Captopril	Metronidazole	**Danazol**	Phenazopyridine
	Chloroquine[S,I]	**Midazolam**[S,I]	**Dapsone**	Phenytoin[S]
	Chlorpheniramine	**Minocycline**	Diclofenac	Piroxicam
	Cyclophosphamide	Misoprostol	Diflunisal	Raloxifene[S]
	Desipramine	**Nifedipine**[S]	Digoxin[S]	**Ritonavir**[S,I]
	Diazepam	Phenobarbital	*Erythromycin*[S,I]	**Saquinavir**[S,I]
	Diltiazem[S,I]	Phenylalanine	Flurbiprofen	**Sirolimus**[S]
	Diphenhydramine	Prednisolone	**Glipizide**	Spironolactone[I]
	Disopyramide	**Primaquine**[S]	Glyburide[S,I]	**Tacrolimus**[S,I]
	Doxepin	Promazine	Griseofulvin	Talinolol[S]
	Doxycycline	Propranolol[I]	Ibuprofen	**Tamoxifen**[I]
	Enalapril	**Quinidine**[S,I]	**Indinavir**[S]	**Terfenadine**[I]
	Ephedrine	Rosiglitazone	Indomethacin	Warfarin
	Ergonovine	Salicylic acid		
	Ethambutol	Theophylline		
	Ethinyl estradiol	Valproic acid		
	Fluoxetine[I]	**Verapamil**[I]		
	Glucose	Zidovudine		
	Imipramine[I]			
Low permeability	**Class 3**		**Class 4**	
	Acyclovir	Fexofenadine[S]	Amphotericin B	
	Amiloride[S,I]	Folinic acid	Chlorthalidone	
	Amoxicillin[S,I]	*Furosemide*	Chlorothiazide	
	Atenolol	Ganciclovir	Colistin	
	Atropine	*Hydrochlorothiazide*	*Ciprofloxacin*[S]	
	Bisphosphonates	Lisinopril	*Furosemide*	
	Bidisomide	Metformin	*Hydrochlorothiazide*	
	Captopril	*Methotrexate*	*Mebendazole*	
	Cefazolin	Nadolol	*Methotrexate*	
	Cetirizine	Pravastatin[S]	Neomycin	
	Cimetidine[S]	Penicillins		
	Ciprofloxacin[S]	Ranitidine[S]		
	Cloxacillin	Tetracycline		
	Dicloxacillin[S]	Trimethoprim[S]		
	Erythromycin[S,I]	Valsartan		
	Famotidine	Zalcitabine		

[a] The listed compounds are predominantly gathered from the literature (1,3–18).

[b] The compounds listed in *italic* are those falling in more than one category by different authors, which could be a result of the definition of the experimental conditions (i.e., acyclovir, amiloride, atropine, and captopril are listed in Classes 1 and 3 but all are highly soluble). Furosemide, hydrochlorothiazide, and methotrexate are listed in Classes 3 and 4, but they are all poorly permeable. Mebendazole is listed as Classes 2 and 4, but the compound is poorly soluble. Interesting examples are ciprofloxacin and erythromycin, which are listed in Classes 2 and 3; it could just be that the properties of the compounds are intermediate between Classes 2 and 3. Ciprofloxacin has also been listed as Class 4.

[c] The compounds listed in bold are primarily CYP3A substrates where metabolism accounts for more than 70% of the elimination; superscript I and/or S indicate P-gp inhibitors and/or substrate, respectively.

science as a whole, exceptions are clues to new discoveries and new hypotheses.

b) The BCS classification criteria for bioequivalence evaluation will not necessarily be appropriate for predicting drug disposition, as mentioned previously for the WHO Essential Medicines List (18), and as will be discussed subsequently.

c) High-permeability drugs are defined as compounds that exhibit 90% absorption in humans following oral dosing according to the FDA BCS criteria (2). Some drugs may fulfill

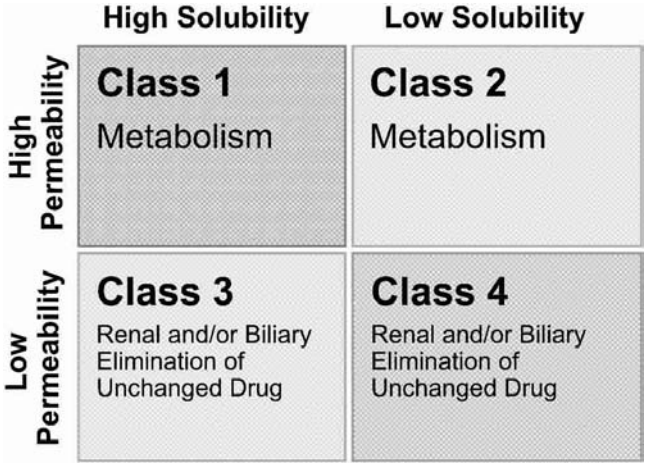

Fig. 2. Predominant routes of drug elimination for drug substances by BCS class.

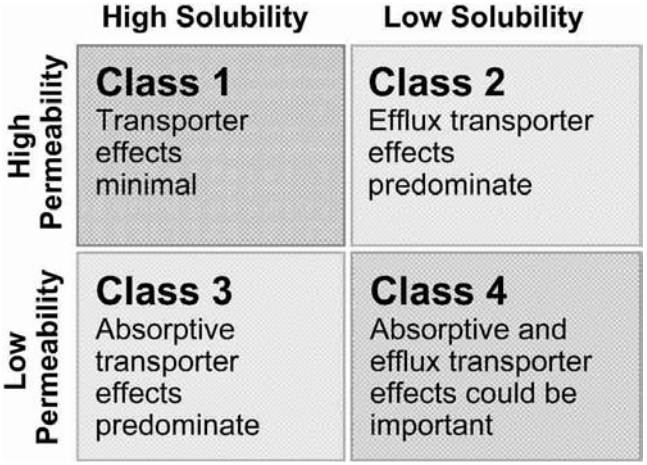

Fig. 3. Transporter effects on drug disposition by BCS class.

these criteria because of the activity *in vivo* of uptake transporters in the intestine, rather than just due to high lipid passive diffusion permeability as reflected in Log P. Thus, some BCS drugs listed in Class 2 (and possibly some Class 1 drugs) may show marked changes in bioavailability when intestinal uptake transporters are inhibited.

d) It is probable that some compounds that should be considered Class 1 in terms of drug absorption and disposition are listed as Class 2 according to the FDA BCS criteria due to the requirement of good solubility and rapid dissolution at low pH values, which is not limiting for drug disposition. This was recently discussed in terms of acidic drugs (26).

We believe that a different set of criteria, particularly those relating to permeability but also to solubility, must be developed when using BCS in predicting drug disposition. We welcome the opportunity to work with the FDA and pharmaceutical manufacturers in setting simple *in vitro* surrogate permeability standards, as we discuss further in the section entitled "Biopharmaceutics Drug Disposition Classification System."

PREDICTING THE EFFECTS OF TRANSPORTERS

Oral Dosing and the Predictability of Transporter Effects

Recent work from our laboratory, initially based on cellular system studies evaluating transporter-enzyme interplay (27–29) have led us to the generalizations regarding transporter effects following oral dosing depicted in Fig. 3. The boldface italic items that follow represent the major predictive generalizations of this section of the current paper.

Transporter effects will be minimal for Class 1 compounds. The high permeability/high solubility of such compounds allows high concentrations in the gut to saturate any transporter, both efflux and absorptive. That is, Class 1 compounds may be substrates for both uptake and efflux transporters *in vitro* in cellular systems under the right conditions [e.g., midazolam (30) and nifedipine (31) are substrates for P-glycoprotein], but transporter effects will not be important clinically. As stated above in Caution d, it is probable that some compounds that should be considered Class 1 in terms of drug absorption and disposition are not Class 1 in BCS due to the requirement of good solubility and rapid dissolution at low pH values. Such pH effects would not be limiting *in vivo* where absorption takes place from the intestine. Examples of this from Table I may include the NSAIDs diclofenac, diflunisal, flurbiprofen, indomethacin, naproxen, and piroxicam, as discussed by Yazdanian *et al.* (26), and warfarin, which is almost completely bioavailable (20,21). In contrast, ofloxacin is listed as Class 2 because of its low solubility at pH 7.5.

Efflux transporter effects will predominate for Class 2 compounds. The high permeability of these compounds will allow ready access into the gut membranes and uptake transporters will have no effect on absorption, but the low solubility will limit the concentrations coming into the enterocytes, thereby preventing saturation of the efflux transporters. Consequently, efflux transporters will affect the extent of oral bioavailability (F_{extent}) and the rate of absorption of Class 2 compounds.

Transporter-enzyme interplay in the intestines will be important primarily for Class 2 compounds that are substrates for CYP3A and Phase 2 conjugation enzymes. For such compounds, intestinal uptake transporters will generally be unimportant due to the rapid permeation of the drug molecule into the enterocytes as a function of their high lipid solubility. That is, absorption of Class 2 compounds is primarily passive and a function of lipophilicity. However, due to the low solubility of these compounds, there will be little opportunity to saturate apical efflux transporters and intestinal enzymes such as CYP 3A4 and UDP-glucuronosyltransferases (UGTs). Thus, changes in transporter expression, and inhibition or induction of efflux transporters will cause changes in intestinal metabolism of drugs that are substrates for the intestinal metabolic enzymes. Note the large number of Class 2 compounds in Table I that are primarily substrates for CYP3A (compounds listed in bold) as well as substrates or inhibitors of the efflux transporter P-glycoprotein (indicated by superscripts S and I, respectively). Work in our laboratory has characterized this interplay in the absorptive process for the investigational cysteine protease inhibitor K77 (28,32) and sirolimus (29), substrates for CYP3A and P-glycoprotein, and more recently for raloxifene (33), a substrate for UGTs and P-glycoprotein.

Absorptive transporter effects will predominate for Class 3 compounds. For Class 3 compounds, sufficient drug

will be available in the gut lumen due to good solubility, but an absorptive transporter will be necessary to overcome the poor permeability characteristics of these compounds. However, intestinal apical efflux transporters may also be important for the absorption of such compounds when sufficient enterocyte penetration is achieved via an uptake transporter.

It has been suggested (Refs. 5, 15, and others in meeting presentations) that products containing Class 3 drug substances should qualify for a waiver of *in vivo* bioequivalence studies on the basis of dissolution studies alone, as for drug products containing Class 1 drugs. This is inappropriate, as it is now obvious that components of a Class 3 drug formulation can affect uptake transporters and modify bioavailability. Until more is known about the importance of intestinal transporters and validated methodology to predict the effects of formulation components on these transporters has been developed, any expansion of *in vivo* bioequivalence study waivers beyond Class 1 compounds is unwise policy. However, our proposal, presented below, could increase the number of drugs that qualify for Class 1 bioequivalence study waivers.

It would be expected that Class 4 compounds could be substrates for both absorptive and efflux transporters. On first principles, we might expect that no Class 4 compounds would become effective drugs due to their solubility and permeability deficiencies. However, it is probable that a number of Class 4 compounds are misclassified in terms of *in vivo* characteristics, as solubility in aqueous solutions may not reflect solubility in gut contents. For example, the FDA generated publication (15) and others have suggested that solubility measurements in surfactant containing solution may be a more appropriate basis for the solubility criteria. For true Class 4 compounds, oral bioavailability is minimal and transporter effects could be relevant, for example, where a change from 2% to 3% bioavailability could make a significant difference.

Food Effects (High-Fat Meals)

It is well-known that food can influence drug bioavailability, both increasing and decreasing the extent of availability (F_{extent}) and the rate of availability. In December 2002, the FDA issued a guidance entitled "Food-Effect Bioavailability and Fed Bioequivalence Studies" (34). Fleisher *et al.* (6) noted that food effects on the extent of bioavailability could generally be predicted based on BCS class, as depicted in Fig. 4. We have added the time to peak exposure (T_{max}) designations to the figure. High-fat meal studies are recommended by the FDA, as such meal conditions are expected to provide the greatest effects on gastrointestinal physiology so that systemic drug availability is maximally affected (34). It is generally believed that food effects result from changes in drug solubility and other factors as listed by the FDA (34), such as food may: "delay gastric emptying; stimulate bile flow; change gastrointestinal pH; increase splanchnic blood flow; change luminal metabolism of a drug substance; and physically or chemically interact with a dosage form or a drug substance." We hypothesize that although these other factors may be important, drug-transporter interactions could often be the primary mechanism for the food effect. We suspect that high-fat meals may inhibit drug transporters, both influx and efflux, and we have carried out preliminary studies that suggest that a high fat meal will inhibit P-glycoprotein (J. M. Custodio and L. Z. Benet, unpublished data).

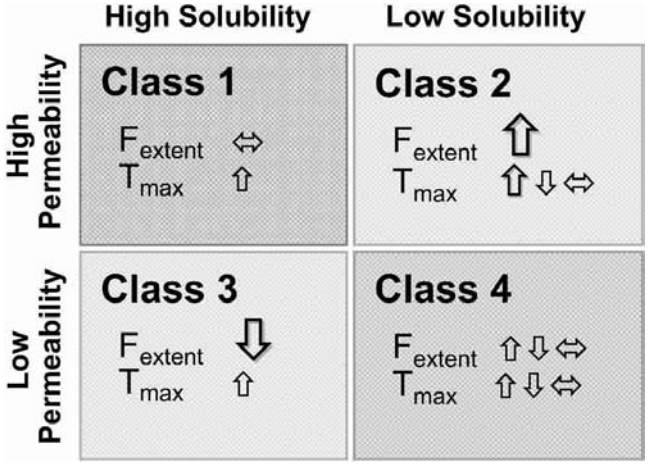

Fig. 4. Predictability of high-fat meal effects by BCS class after Fleischer *et al.* (6).

High-fat meals will have no significant effect on F_{extent} for Class 1 compounds because complete absorption may be expected for high solubility/high permeability compounds, and as noted previously, no transporter drug interactions would be expected for Class 1 compounds.

However, high-fat meals may delay stomach emptying and therefore cause an increase in peak time.

High-fat meals will increase F_{extent} for Class 2 compounds due to inhibition of efflux transporters in the intestine and additional solubilization of drug in the intestinal lumen (e.g., micelle formation). Peak time could decrease due to inhibition of efflux cycling or increase due to slowing of stomach emptying; a combination of the two will usually be dominated by the delayed emptying. This will be true in cases where membrane permeation is passive, such as for the immunosuppressants cyclosporine, tacrolimus, and sirolimus. However, if high permeability for a Class 2 compound results from uptake transporters, rather than ready partition into the intestinal membranes (see Caution c above), high-fat meals could inhibit both uptake and efflux transporters. Then, depending upon the relative magnitude of inhibition of uptake and efflux transporters, meal effects may be confounding, more likely having little effect on F_{extent}, but still increasing peak time due to delayed gastric emptying.

Formulation changes that markedly increase the solubility of Class 2 compounds will decrease or eliminate the high-fat meal effects for these drugs. We believe that this is the reason that the newer cyclosporine microemulsion formulation (Neoral) eliminates the food effects associated with the older olive oil formulation (Sandimmune). In practice, drug formulators attempt to enable a Class 2 compound to function as a Class 1 compound, thereby eliminating food effects on F_{extent} and other transporter-drug interactions, as explained earlier for Class 1 drugs.

High-fat meals will decrease F_{extent} for Class 3 compounds due to inhibition of uptake transporters in the intestine. Recent evidence suggests that intestinal drug uptake can be decreased by inhibiting organic anion transporting polypetides, as shown by the effect of fruit juices on fexofenadine (35). As noted above, some Class 3 compounds can be substrates for intestinal efflux transporters. Depending upon whether the meal effects are more pronounced on efflux or

influx transporters for a Class 3 drug that is a substrate for both, an unexpected increase in the extent of bioavailability or no meal effect may be observed. We hypothesize that this may be the explanation for the lack of a high-fat meal effect on acyclovir. For Class 3 drugs, peak time would be expected to increase by a high-fat meal due to the combination of delayed stomach emptying and slower absorption.

For Class 4 compounds, it is difficult to predict what will occur, as all of the interacting effects mentioned for Class 2 and Class 3 compounds can be seen here. However, although not shown in Fig. 4, we believe that if high-fat meal effects are to occur, an increase of F_{extent} is more likely, resulting from the combination of increased solubilization of drug in the intestine and inhibition of efflux transporters.

Postabsorption Effects and Intravenous Dosing

For intravenous dosing, drug concentrations at the eliminating organ will always be relatively low due to the diluting effects of volume of distribution, as compared to concentrations of drug in the intestine. Therefore, saturation of transporters (and enzymes) will be minimal, if at all, and solubility considerations will be unimportant when measurable systemic concentrations of the drug are achieved.

High extraction ratio drugs, where clearance approaches blood flow, are mainly limited to Class 1 compounds (and possibly a few Class 2 compounds so designated because of poor solubility at low pH; see Caution d). This will be true because the metabolism of such drugs is not rate limited either by dissolution or permeability. Examining Table I compounds with respect to pharmacokinetic data (20,21) reveals that 16 Class 1 compounds exhibit total clearance greater than half of liver blood flow (>10.7 ml min^{-1} kg^{-1}) (abacavir, amitryptyline, buspirone, captopril, diltiazem, doxepin, imipramine, isosorbid dinitrate, labetolol, levodopa, metoprolol, meperidine, misoprostol, propranolol, verapamil, and zidovudine), whereas only four Class 2 compounds (haloperidol, indinavir, itraconazole, and raloxifene), one Class 3 (pravastatin), and one Class 4 (mebendazole—probably misclassified) compound meet this criterion (Caution a).

Post intestinal absorption and following intravenous dosing, both uptake and efflux transporters can be important determinants of the disposition for Classes 2, 3, and 4 compounds. They will also be important for Class 1 compounds where high permeability results from uptake transporters (Caution c). Recent work in our laboratory has evaluated the importance of the rat hepatic uptake transporter oatp2 for digoxin (36,37), erythromycin (38), and atorvastatin (39). Using the rat isolated perfused liver, we were not able to demonstrate a significant role for this transporter in the hepatic uptake of cyclosporine, dantrolene, nelfinavir, saquinavir, simvastatin, and talinolol (Y. Y. Lau, H. Okochi, N. Watanabe, and L. Z. Benet, unpublished results). These studies emphasize the difficulty in presenting a simple generalized conclusion (Caution a) about the importance of uptake transporters in determining the disposition of the highly permeable Class 2 compounds. Obviously, there will be gradations within each broad BCS class for the permeability and solubility parameters (16,40). The difference observed here between the importance of an uptake transporter for atorvastatin vs. simvastatin, two HMG-CoA reductase inhibitors, is most likely related to the differences in lipophilicity (often reflective of pK_a) at the biologically relevant pH [simvastatin Log P = 4.42, Log D (pH 7.0) = 4.41 vs. atorvastatin Log P = 4.23, Log D (pH 7.0) = 1.54]. That is, at a pH close to physiologic, simvastatin is much more permeable than atorvastatin. It is more obvious that both uptake and efflux transporters will be involved in determining the disposition characteristics for Class 3 and Class 4 compounds as demonstrated by the recent double transfected cellular system studies reported by the Sugiyama and Kim groups investigating the importance of both uptake and efflux transporters on pravastatin (41) and fexofenadine (42).

Biliary secretion of parent drug can be an important component of disposition for Classes 3 and 4 compounds. Biliary secretion of most Classes 1 and 2 parent drugs will be negligible due to extensive metabolism, although biliary excretion of metabolites can be important.

Renal elimination of Classes 3 and 4 compounds can be affected by both uptake and efflux transporters. Furthermore, metabolism of Classes 3 and 4 compounds in the kidney, and transporter-enzyme interplay, may be important for drugs where a kidney (vs. liver) specific uptake transporter is involved (e.g., furosemide). Metabolism of Class 2 (and possibly Class 1) compounds can be important in the kidney, when a kidney-specific enzyme such as CYP3A5 is identified (e.g., tacrolimus and cyclosporine).

Attempts to use markers of enzymatic processes (e.g., midazolam vs. erythromycin breath test) to predict metabolism of another substrate cannot be expected to work when the test drugs are in different BCS classes. Even when two enzymatic substrates are in the same class, there is little chance to detect a potential correlation when the two test compounds are substrates for different uptake and efflux transporters. Many, many papers have investigated the potential for one substrate to predict the metabolism of other substrates by the same enzyme. Almost all of these attempts have failed, and we believe that the reason for the lack of correlation is due to differences in transporter susceptibilities. For example, erythromycin is a substrate for both uptake and efflux transporters as well as of CYP3A4. It is obvious that the ability of erythromycin metabolism to predict the metabolism of other CYP3A4 compounds will be compromised if differences in transport are not identified and fully taken into account. We believe, at this time, administration of "cocktails" of substrates (i.e., a mixture of small quantities of drugs that are specific substrates for particular metabolic enzymes) to characterize a patient's metabolic potential will be of little use, except for the most obvious pharmacogenetic differences in enzyme capacity.

Drug-Drug Interactions

Drug-drug interactions are not limited to enzymatic processes but can frequently be mediated by transporter interactions and often involve transporter-enzyme interplay for Class 2 compounds. Using our CYP3A4 transfected Caco-2 cellular system (27), we demonstrated that for flux in the apical to basolateral direction, inhibition of P-glycoprotein caused a decrease in the extraction ratio of K77 (28) and sirolimus (29), both substrates for CYP3A4 and P-glycoprotein, although under the same conditions there was

no change in the extraction ratio for felodipine and midazolam, substrates only for CYP3A4 in this cellular system. In the isolated perfused rat intestine, where drug flux is in the same direction, we confirmed that a similar decrease of the intestinal extraction ratio is seen for K77 when only P-glycoprotein was inhibited (32). That is, inhibition of the intestinal efflux transport changed intestinal metabolism even though the inhibitor had no direct effect on the enzyme itself.

In contrast, for basolateral to apical flux in the cellular system, inhibition of P-glycoprotein resulted in increased metabolism of K77 and sirolimus, again with no effects seen on felodipine and midazolam (28,29). We demonstrated a similar result using the isolated perfused liver, where inhibition of P-glycoprotein caused increased clearance of tacrolimus (43). Again, in the isolated perfused rat liver we demonstrated that inhibition of P-glycoprotein increased digoxin clearance and increased formation of its primary metabolite, digoxigenin bisdigitoxoside (36). The same result was observed in freshly isolated rat hepatocytes (37). That is, in the liver, inhibition of the efflux transporter P-glycoprotein caused increased metabolism even though the inhibitor showed no activating effects on the enzyme. We also demonstrated in the isolated perfused liver and hepatocyte studies that inhibition of the uptake transporter oatp2 caused an increase in digoxin perfusion concentrations and a decrease in the formation of the primary metabolite relative to control (36,37). These studies demonstrate that transporter inhibition can occur at inhibitor concentrations that have been found to be relevant for enzyme inhibition.

In general, in vitro microsomal studies that show metabolism changes for a drug when an interacting substrate is added will be predictive of an in vivo interaction, but will not necessarily yield a quantitative prediction. However, when an in vitro microsomal study shows no metabolic interaction, it cannot be concluded that an in vivo metabolic interaction will not occur, particularly for Class 2 compounds where transporter-enzyme interplay can result in significant metabolism changes due to transporter inhibition. Our major difference with the positions of the FDA guidance in this area (44) and the PhRMA Drug Metabolism and Clinical Pharmacology Technical Working Groups (45) is that lack of an *in vitro* drug-drug metabolic interaction cannot assure that an *in vivo* metabolic interaction will be absent, particularly for Class 2 compounds. Thus, we are suggesting that the major time and money saving result from *in vitro* drug-drug metabolic interaction studies, that is the ability to rule out the need for an *in vivo* drug-drug interaction study, may not be justified. We hope that this manuscript will shed further light on the methodology or requirements necessary to evaluate drug-drug interactions when transporter involvement is likely.

Following oral dosing, major significant interactions will occur for Class 2 drugs that are substrates for both intestinal enzymes (e.g., CYP3A, UGTs) and intestinal apical efflux transporters (e.g., P-glycoprotein, MRP2, BCRP). This is because concomitant inhibition of the intestinal enzymes and the apical efflux transporter both lead to less gut metabolism in a way that can synergistically increase systemic drug concentrations (Table II). It is, therefore, not surprising that drugs removed from the market at FDA's recommendation due to drug-drug interactions are predominately orally-dosed drugs that are substrates for both CYP3A and P-glycoprotein (46).

The enzyme-efflux transporter interplay that is so important in the intestine will not be as significant in the liver (and the kidney) due to the reverse order in which drug molecules encounter the two proteins. As we recently summarized (47), in the intestine an absorbing drug encounters the apical efflux transporter first and then the enzyme, so that inhibition of the efflux transporter decreases access to the enzyme by preventing recycling (Fig. 5). In contrast in the liver (or kidney), the drug molecule encounters the enzyme prior to the apical efflux transporter (Fig. 5). Therefore, inhibition of the apical efflux transporter increases access to the enzyme and increases the extent of metabolism by the active enzyme (36,37,43). Thus, inhibition of both the enzyme and apical efflux transporter in the liver (or kidney) will have opposing effects, decreased enzyme activity but increased exposure to the enzyme due to the inhibition of the apical efflux transporter (Table II). We demonstrated this in our rat liver perfusion study of tacrolimus using equipotent inhibitory concentrations of cyp3a for troleandomycin and cyclosporine (43). Because cyclosporine inhibits both cyp3a and P-glycoprotein, the area under the curve for tacrolimus in the liver perfusion studies was significantly greater when troleandomycin (cyp3a inhibitor only) was used as an inhibitor rather than cyclosporine, due to the counteracting effects depicted in

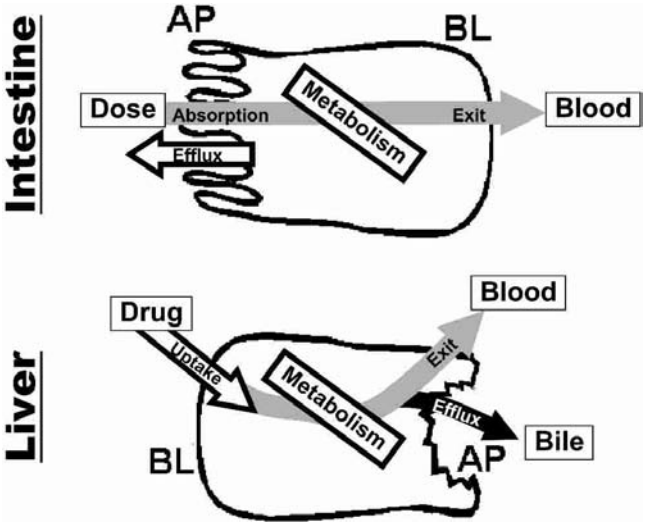

Fig. 5. The relationship between metabolic enzymes and transporters in the intestine and liver after Benet *et al.* (47). In the intestine, the efflux transporter on the apical border is anterior to the metabolic enzymes. Therefore, inhibition of the efflux transporter decreases drug access to the enzyme by preventing recycling and speeds the rate of absorption, thereby facilitating enzyme saturation. In contrast, in the liver the drug encounters the enzyme prior to the efflux transporter, and inhibition of the apical transporter increases drug access to the enzyme. In the intestine, inhibition of apical efflux and metabolism are synergistic, both increasing systemic AUC (Table II). In the liver, inhibition of the basolateral uptake transporter and metabolism are synergistic, while inhibition of apical efflux and metabolism yield opposing effects (Table II). Although apical uptake transporters are present in the intestine, their relevance for uptake of highly lipophilic Classes 1 and 2 drugs *in vivo* has not been demonstrated, and we have omitted them from this figure and Table II. Basolateral efflux transporters in the liver may exist but their *in vivo* relevance has not been confirmed, although we speculate the outcome of inhibition in Table II as the outcome is the same for the inhibition of either the apical or basolateral efflux transporters.

Table II. Predicted Direction of Change for Systemic Exposure (AUC) of Class 2 Drugs Resulting from Inhibition of Relevant Enzymes and Transporters in the Intestine and Liver

	Intestine		Liver	
	Efflux	Absorptive efflux	Efflux	Influx
	Apical	Basolateral	Apical/basolateral	Basolateral
Transporter inhibited	⇧	⇩	⇩	⇧
Enzyme inhibited	⇧	⇧	⇧	⇧
Enzyme + transporter inhibited	⇧⇧	⇔⇩⇧	⇔⇩⇧	⇧⇧

Table II that would be expected by the addition of cyclosporine. In contrast, when felodipine was the substrate, no difference in drug concentrations was observed whether troleandomycin or cyclosporine was the inhibitor, since felodipine is not a substrate for the efflux transporter (43).

The increase in hepatic metabolism due to inhibition of hepatic efflux transporters may have implications for the high fat meal effects observed with Class 2 compounds, as discussed earlier. In the early 1990s, we, as well as others, demonstrated a marked high-fat-meal-associated increase in area under the curve (AUC) for the Sandimmune formulation of cyclosporine. We observed that in healthy volunteers a high fat meal increased blood AUC 76 ± 18% relative to a low fat control meal (48). We also carried out a study to evaluate the effects of a high fat meal on an intravenous Sandimmune formulation and observed a significant ($p < 0.002$) increase in cyclosporine clearance (49), an unexpected result that we were previously unable to explain adequately. Having recently carried out preliminary studies showing that a high-fat meal can inhibit P-glycoprotein (J. M. Custodio and L. Z. Benet, unpublished data), we propose that the increase in cyclosporine clearance with a high fat meal is due to inhibition of hepatic efflux (Table II). This may also explain why, for some Class 2 compounds, a high-fat meal may not increase F_{extent}, or more correctly AUC, as inhibition of P-glycoprotein in the intestine and liver will have opposing effects (Table II).

Inhibition of hepatic uptake transporters can lead to significantly increased systemic drug concentrations for Class 2 compounds that will not be predictable from in vitro microsomal metabolic interaction studies. Here, inhibition of hepatic enzymes and basolateral influx transporters will yield synergistic increases in systemic concentrations (Fig. 5 and Table II). Our recent *in vitro* studies using rat hepatocytes as a model to explain the effect of renal failure on hepatic elimination of erythromycin showed that one uremic toxin, indoxyl sulfate, had the potential to inhibit cyp3a, while a second uremic toxin, CPMF, was a potent inhibitor of the hepatic uptake transporter oatp2 (38).

As described here, we believe that transporter-enzyme interplay will only be significant for Class 2 compounds and that intestinal absorptive influx transporters will not be relevant for such compounds. Therefore, in Table II and Fig. 5 we do not include any potential for interplay between intestinal apical influx transporters and intestinal metabolic enzymes. Note also in Table II that inhibition of a hepatic efflux transporter is predicted to yield the same effect, independent of whether the transporter is located on the apical or basolateral border of the hepatocyte. Confirming experimental evidence for the effect of inhibition of a hepatic basolateral efflux transporter has yet to be published, and therefore we have not depicted such a transporter in Fig. 5.

Inhibition of hepatic and renal uptake transporters can lead to significant increases in the systemic concentration of Classes 3 and 4 compounds. We expect these interactions to be of reduced magnitude compared to those potentially involving transporter-enzyme interplay for Class 2 compounds, where the possibility of a synergistic inhibitory effect on enzymes and transporters can occur (Table II).

Drug-drug interactions for Class 1 compounds will be primarily metabolic, with transporter-enzyme interplay only becoming important for those drugs where high permeability is a result of rapid transporter uptake rather than high Log P (Caution c).

We suspect that some drug-drug interactions, previously attributed to pH changes or intestinal transit time changes, particularly for Class 3 compounds, may prove to be transporter-mediated.

BIOPHARMACEUTICS DRUG DISPOSITION CLASSIFICATION SYSTEM

The development of the BCS was a major step in bringing rational science to regulation, allowing waivers of *in vivo* bioavailability and bioequivalence testing ("biowaiver") of immediate release dosage forms for high-solubility, high-permeability drugs when such drug products also exhibited rapid dissolution (1,2). This application of science yielded a decrease in the regulatory burden. When the BCS was first developed there was only a nascent understanding of the importance of drug transporters to bioavailability. However, as pointed out here, for Class 1 compounds neither efflux nor absorptive transporters should influence oral bioavailability, and meal effects on F_{extent} should be negligible. Thus, we believe that there is now little need for concern about any effects of excipients contained in Class 1 drug products on the extent of bioavailability.

The FDA has suggested a variety of possible biowaiver extensions (15). We would support reducing the high-permeability requirement from 90% to 85% absorbed but propose an even further expansion below. We are concerned with possible biowaiver extensions to compounds of BCS Classes 2, 3, and 4 compounds as we have stated above. With respect to Class 3 drugs, the argument has been made that as the FDA does not require bioequivalence of solution formulations, it appears to be logical to extend biowaivers to Class 3 drug products where solubility of the drug substance is not a concern. However, when a solid dosage form is manufac-

tured excipients must be added and these excipients can affect uptake transporters. Yu *et al.* (15) have suggested that such waivers could be justified when immediate release products "contain only known excipients that do not affect the oral drug absorption." As pointed out above, we believe that it is too early to make such a general biowaiver decision concerning the importance of excipient effects on uptake transporters. We agree that it may be possible for the FDA to list certain Class 3 drugs and certain excipients as qualifying for a waiver of *in vivo* bioequivalence. However, this potential expansion does not have the same regulatory impact or influence as the present BCS regulation, which holds for a potentially large group of Class 1 substances.

Designation of the major route of drug elimination as part or instead of the permeability criteria (as shown in Fig. 6) would reduce the regulatory burden for many more Class 1 compounds, would eliminate the ambiguity and difficulty in determining 90% (or 85%) absorption for Classes 1 and 2 compounds, and would allow predictability of absorption and disposition characteristics of drugs in all four Biopharmaceutics Drug Disposition Classification System (BDDCS) classes, as detailed in the 20 bold italic generalization in the previous section of this paper. As pointed out by many investigators, although there are some difficulties in differentiating solubility classes, the major uncertainty relates to the permeability assignment. Thus, we propose that it may be more useful to replace the permeability criterion with the major route of drug elimination in assigning drugs to BDDCS classes. As we note in Fig. 6, BDDCS Class 1 compounds would then be designated as "high solubility, extensive metabolism." Waiver of *in vivo* bioequivalence studies for BDDCS Class 1 drugs would still require rapid dissolution. BDDCS Class 2 compounds would be designated as "poor solubility, extensive metabolism" drugs, BDDCS Class 3 as "high solubility, poor metabolism," and BDDCS Class 4 as "low solubility, poor metabolism." In Table I, under BDDCS criteria, the dually listed BCS Class 1 and Class 3 compounds acyclovir, amiloride, atropine, and captopril would all be BDDCS Class 3; mebendazole would be BDDCS Class 2; erythromycin would be BDDCS Class 3; ciprofloxacin would be BDDCS Class 3 or Class 4; and digoxin, ofloxacin, phenazopyridine, and talinolol would become BDDCS Class 4. Note in Table I that we and others have listed the HIV protease inhibitors indinavir, nelfinavir, ritonavir, and saquinavir as BCS Class 2, whereas Lindenberg *et al.* (18) listed these four compounds as BCS Class 4. These drugs illustrate why we prefer the BDDCS classification as in Fig. 6. Any classification system should serve to increase predictability. A main purpose of this paper is to detail the potential for predicting the effects of transporters on drug disposition as we have described in the points earlier in this manuscript. We believe, in general, that the predictability for the disposition of these protease inhibitors is more relevant if they are categorized as BDDCS Class 2 compounds rather than BCS Class 4 (although we do recognize the anomalously high clearance value for indinavir and its decreased F_{extent} with high-fat meals; see Caution a).

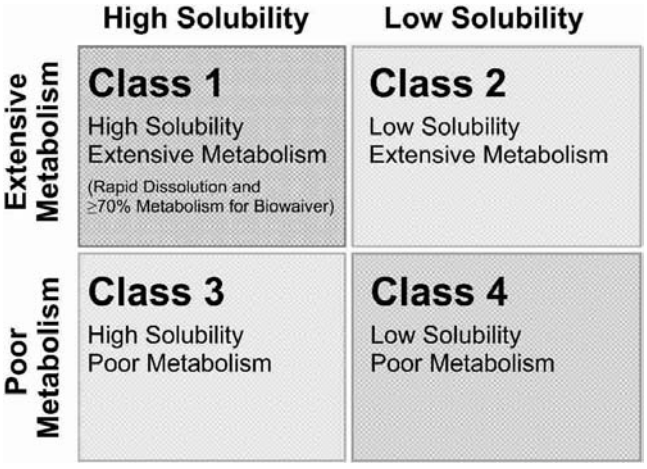

Fig. 6. The Biopharmaceutics Drug Disposition Classification System (BDDCS) where major route of elimination (metabolized vs. unchanged) serves as the permeability criteria.

We find it inconsistent to suggest that products containing BCS Class 3 drugs should qualify for a waiver of *in vivo* bioequivalence studies on the basis of dissolution studies (5,15), when a large number of BCS Class 1 drugs cannot now qualify for the waiver due to the difficulty of proving that the drug is 90% (or 85%) absorbed. As a first defining "extensive metabolism" criterion (Fig. 6) for classifying a drug as BDDCS Classes 1 or 2, we propose ≥50% metabolism of an oral dose *in vivo* in humans, but believe that upon further analysis this breakpoint might change ±10%. However, because this is a new approach, we propose that initially the criteria for waiver of *in vivo* bioequivalence for BDDCS drugs be high solubility, rapid dissolution (as per BCS Class 1, retaining the pH 1–7.5 requirement) and ≥70% metabolism of the active drug, as depicted in Fig. 6. Of the compounds solely listed as Class 1 in Table I, the only drugs that do not meet the ≥70% (or the ≥50%) criteria are chloroquine, doxycycline, ephedrine, ethambutol, levofloxacin, and lomefloxacin, and under BDDCS these drugs should be listed as Class 3 and thus would not be eligible for a waiver of *in vivo* bioequivalence studies at this time.

We believe, it will be easier and less ambiguous to determine the assignment of BDDCS classes based on the extent of metabolism than using permeability (i.e., extent of absorption) in BCS assignments. As recognized by all investigators in the field, permeability assignment is uncertain and difficult to perform, as permeability is based on an absorption measure in humans, not bioavailability, and for most drugs data following intravenous dosing in humans are not available. In contrast, the BDDCS extent of metabolism criterion (≥50% or ≥70% of the oral dose) is relatively easy to quantify using the modern analytical methodology routinely used in drug development. This is exemplified by our designation of BDDCS class for 168 drugs/compounds in Table III. Here, we are able to remove the ambiguities (double/triple listings) in Table I utilizing the solubility determinations of Lindenberg *et al.* (18), in most cases, and the extent of metabolism values from the literature (Refs. 20 and 21 and original sources). This allowed us to change the categorization for 10 drugs in Table I, as we have discussed above, eliminate multiple categorization for 11 drugs and add, with confidence, 38 additional compounds. Because a major purpose of this paper is to facilitate predictability of transporter-enzyme interplay and the potential for drug-drug and disease-drug interactions for particular substrates, in Table III we list in bold those compounds in Classes 1 and 2 that may be expected to exhibit significant first-pass intestinal metabolism. We designate with

a superscript "U" those drugs that have been identified as substrates for uptake transporters. As would be expected, a proportionally greater fraction of drugs that are substrates for uptake transporters is found for Class 3 and Class 4 compounds, as compared to the Classes 1 and 2 drugs.

A further advantage of BDDCS is that a preliminary class assignment for NMEs may be obtained from a metabolism measure in human hepatocytes, prior to *in vivo* studies in humans. As discussed above, this determination must be made in cellular systems that preserve the relationship of uptake and efflux transporters with metabolic enzymes (i.e., microsome studies would not be sufficient to determine the assignment of BDDCS class) (37). We would welcome the opportunity to work with pharmaceutical companies and the FDA, who are in possession of large databases of such hepatocyte study results that can be used to define the relevant parameters. Note that the parameter needed here is not the metabolic clearance that can be predicted from *in vitro* measures of intrinsic clearance, protein binding, and blood flow, but rather the fraction of the total clearance that is metabolic, a parameter not previously well characterized from *in vitro* studies. Complete metabolic elimination of drug can occur for substances exhibiting very low clearance (e.g., warfarin).

One reviewer of this manuscript raised the following issue, which because of its importance we quote here and respond. The reviewer states:

"The rationale to use Permeability and Solubility as parameters in BCS for Biowaiver of a BE study is derived from the fact that these two factors directly determine the oral absorption profile of drugs, thus IVIVC of BA/BE can be discussed by using these parameters. As the authors have pointed out in this paper, the new parameter, the extent of metabolism, might have a good relation with drug permeability. However, still there is no guarantee that drugs metabolized more than 70% of dose always show high-permeability to the intestinal membrane. Extent of metabolism is not a direct parameter to define the drug absorption. From a scientific standpoint, I recognize the importance of the new classification system, but as a regulatory application, I cannot agree to use this new system for the present."

We do not expect any rapid acceptance of our ideas from a regulatory perspective. We recognize that 5 years elapsed between the initial report of Amidon *et al.* (1) and the FDA BCS guidance (2). And we have no objection to retaining the current requirements for Class 1 compound biowaivers, with the recognition that many eligible drugs will be excluded due to the difficulty of proving 90% (or 85%) absorption. However, it is very obvious that the knowledgeable scientific community in this field does not believe that a permeability criterion should be restrictive of drugs for which drug products are eligible for a biowaiver. As discussed above, there is much support for allowing products containing Class 3 drug substances to qualify for a waiver of *in vivo* bioequivalence studies on the basis of dissolution criteria only (5,15). This was the opinion of the AAPS consensus workshop (50) and is recommended in the WHO Working Document QAS/04.093 (51). Therefore, we are not concerned that our metabolism criterion for highly soluble compounds will create difficulties if appropriate dissolution criteria are implemented, as proposed in the WHO Working Document.

Of course, it would be even simpler to assign correctly the classes in Fig. 6 if an *in silico* methodology were validated.

Development of a Relationship Between Disposition and Permeability

The Lipinski Rule of 5 (22–24) was an attempt to define upper limits of lipophilicity for developing "drugable" compounds. From the material presented here, it is obvious that a disposition permeability relationship (DPR) should be investigated and defined to characterize the crucial border between BDDCS Classes 1 and 2 compounds that are highly metabolized vs. BDDCS Classes 3 and 4 compounds that are primarily eliminated unchanged, and that such a relationship would be a very useful addition to the discovery and development of therapeutic agents. We anticipate that the DPR will have components related to physicochemical parameters [such as Lipinski Rule of 5 characteristics and parameters related to polar surface area and/or molecular flexibility (22,23,52)], together with qualifications related to uptake (and efflux) transporters, and a parameter related to the extent of metabolism.

In 2002, Mandagere *et al.* (53) reported their attempt to combine permeability measures with the fraction of drug not metabolized in 30 min in incubations with hepatic microsomes or S9 fractions to predict *in vivo* bioavailability. They reported some success but suggested that the model's predictability "is best applied to passively diffused compounds, which accounts for approximately 80% of all compounds." The seven identified drugs plus mannitol in their data set included Class 1 high extraction ratio drugs (metoprolol, propranolol, and verapamil), which of course would be expected to show low bioavailability; BDDCS Class 2 intermediate to low extraction ratio compounds (indomethacin, carbamazepine and warfarin), which would be expected to show intermediate to high bioavailability; timolol (probably BDDCS Class 2), a compound with higher clearance than the other Class 2 compounds, but less than the Class 1 compounds, and therefore expected to have intermediate bioavailability among these 7 drugs; and finally only one unmetabolized substance, mannitol (probably BDDCS Class 3), with very poor permeability, that would be expected to show poor bioavailability. So in essence, this report (53) just confirms the finding of Smith (19) that more permeable lipophilic compounds make good substrates for CYP enzymes, as the fraction not metabolized in 30 min of incubation is a measure of clearance, but the method will not be able to account for differences in bioavailability for Class 2 compounds that result from transporter-enzyme interplay, or for Class 3 drugs where uptake transporters will be the defining determinant of bioavailability. As outlined here, incorporation of recent scientific understanding should allow the pharmaceutical sciences community to develop a DPR parameter with predictability [i.e., it is not true that permeability of 80% of all compounds is due to passive diffusion; hepatocytes (or some other system that maintains the architecture of transporters and enzymes) should be used for the metabolism studies, not microsomes or the S9 fraction, so to include transporter-enzyme interplay; the fraction of total clearance that is attributable to metabolism rather than the metabolic clearance is the "permeability" parameter that differentiates BDDCS classes; both influx and efflux transporters must be considered; although clearance is a reasonable predictor of bioavailability for Class 1 compounds, this will often not be true for drugs from Classes 2, 3 and 4 where transporters cause differential effects between

Table III. Biopharmaceutics Drug Disposition Classification System (BDDCS)[a]

	High solubility		Low solubility	
Extensive metabolism	**Class 1**		**Class 2**	
	Abacavir	**Ketorolac**	Albendazole	**Ketoconazole**[I]
	Albuterol[b]	Ketoprofen	**Amiodarone**[I]	**Lansoprazole**[I]
	Acetaminophen	Labetolol	**Atorvastatin**[S,I]	Lopinavir
	Allopurinol	Levamisole	Azathioprine	**Lovastatin**[S,I]
	Amitryptyline[S,I]	Levodopa[S,U]	**Azithromycin**[S,I]	Mebendazole
	Antipyrine	**Lidocaine**[I]	**Carbamazepine**[S,I]	**Mefloquin**[S,I]
	Buspirone	**Meperidine**	**Carvedilol**	Nalidixic acid
	Caffeine	Metoprolol	Chlorpromazine[I]	Naproxen[U]
	Chloramphenicol	Metronidazole	**Cisapride**[S]	Nelfinavir[S,I]
	Chlorpheniramine	**Midazolam**[S,I]	**Clofazamine**	**Nevirapine**
	Codeine	**Minocycline**	**Cyclosporine**[S,I]	Oxaprozin
	Colchicine[S]	Misoprostol	**Danazol**	Phenytoin[S]
	Cyclophosphamide	Morphine[S]	**Dapsone**	Piroxicam[U]
	Desipramine	**Nifedipine**[S]	Diclofenac	Praziquantel
	Dexamethasone[S]	Phenobarbital	Diflunisal	Raloxifene[S]
	Diazepam	Phenylalanine	**Efavirenz**[I]	**Rifampin**[S,U]
	Diltiazem[S,I]	Prednisolone	Flurbiprofen	**Ritonavir**[S,I]
	Diphenhydramine	**Primaquine**[S]	Glipizide	**Saquinavir**[S,I]
	Disopyramide	Promazine[S]	**Glyburide**[S,I]	**Sirolimus**[S]
	Doxepin	**Promethazine**[S]	Griseofulvin	Spironolactone[I]
	Enalapril[U]	Propranolol[I]	**Haloperidol**[S,I]	Sulfamethoxazole
	Ergonovine	Pyranzinamide	Ibuprofen	**Tacrolimus**[S,I]
	Ergotamine	**Quinidine**[S,I,U]	**Indinavir**[S]	**Tamoxifen**[I]
	Ethinyl estradiol[S]	**Quinine**[S,I,U]	Indomethacin[U]	**Terfenadine**[I]
	Fluoxetine[I]	Rosiglitazone	**Itraconazole**[S,I]	Warfarin
	Glucose	Salicyclic acid[U]	Ivermectin[S,I]	
	Hydralazine	Theophylline		
	Imipramine[I]	Valproic acid		
	Isoniazid	**Verapamil**[I,U]		
	Isosorbid dinitrate	Zidovudine[U]		
Poor metabolism	**Class 3**		**Class 4**	
	Acyclovir[U]	Folinic acid	Acetazolamide[U]	
	Amiloride[S,I]	Ganciclovir[U]	Aluminum hydroxide	
	Amoxicillin[S,I,U]	Hydrochlorothiazide	Amphotericin	
	Atenolol	Lamivudine	Chlorthalidone	
	Atropine	Levofloxacin[S]	Chlorothiazide	
	Bisphosphonates	Lisinopril[U]	Ciprofloxacin[S]	
	Bidisomide	Lithium	Colistin	
	Captopril[U]	Lomefloxacin	Digoxin[S,U]	
	Cefazolin[U]	Metformin[U]	Furosemide[U]	
	Cetirizine	Methotrexate[U]	Neomycin	
	Chloroquine[S,I]	Metoclopramide	Nystatin	
	Cimetidine[S,U]	Nadolol	Ofloxacin	
	Ciprofloxacin[S]	Neostigmine	Phenazopyridine	
	Cloxacillin	Pravastatin[S,U]	Talinolol[S,U]	
	Dicloxacillin[S]	Penicillins[U]		
	Doxycycline	Pyridostigmine		
	Ephedrine	Ranitidine[S,U]		
	Erythromycin[S,I,U]	Riboflavin		
	Ethambutol	Tetracycline		
	Famotidine[U]	Trimethoprim[S]		
	Fexofenadine[S,U]	Valsartan		
	Fluconazole[I]	Zalcitabine[U]		

[a] Solubility classification was predominantly gathered from the literature (1,3–18). Metabolic classification was based on literature data (20, 21) and original sources with ≥50% being defined as extensive metabolism.

[b] The compounds listed in bold may be expected to exhibit significant intestinal first-pass metabolism because they are primarily CYP3A substrates, or substrates for intestinal UGTs (raloxifene) or intestinal sulfotransferases (albuterol); superscript I and/or S indicate P-gp inhibitor and/or substrate, respectively; superscript U indicates substrate for an uptake transporter. Transporter susceptibility was taken from original sources.

gut and liver]. Again, we encourage others to pursue such an analysis, and would welcome the opportunity to collaborate in investigating this relationship.

CONCLUSIONS

During the past few years, studies in our laboratory of transfected cellular systems, isolated perfused rat livers and intestines, and studies in primary hepatocytes have led us to a better understanding of the interplay between transporters, both influx and efflux, and metabolic enzymes in the intestine and the liver. We recognize that this interplay could differ depending on the drug's solubility and permeability characteristics as reflected in the Biopharmaceutical Classification System (BCS). Although BCS has had a marked effect in decreasing the regulatory burden by allowing a waiver of *in vivo* bioequivalence studies for a limited number of Class 1 drugs, little predictive use has been made of Classes 2, 3, and 4 in the BCS categorization. We noted that, in general, BCS Classes 1 and 2 are highly metabolized, whereas BCS Classes 3 and 4 drugs are primarily excreted unchanged via the biliary or renal routes. We therefore suggest that changing the permeability component to a route of elimination component in a Biopharmaceutics Drug Disposition Classification System (BDDCS) will facilitate predictions, markedly expand the number of Class 1 drugs eligible for waiver of *in vivo* bioequivalence studies, and provide new insight. We detail how such a classification system can be used in categorizing routes of elimination; predicting the effects of efflux and absorptive transporters on drug absorption; predicting when transporter-enzyme interplay will yield clinically significant effects; predicting the direction and importance of food effects; predicting transporter effects following intravenous dosing and on post absorption systemic levels; and in defining drug-drug interaction potential. We point out where inhibiting both enzymes and transporters can have synergistic effects and where it can have opposing effects. Finally, we suggest that it may be easier to determine classification based on major routes of elimination than upon permeability; we propose an extent of metabolism criterion for waiver of *in vivo* bioequivalence studies; and we suggest how predictive algorithms may be developed using only *in vitro* or *in silico* methods to facilitate class assignment in BDDCS.

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