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The Use of Drug Metabolism for Prediction of Intestinal Permeability†

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Abstract: The Biopharmaceutics Classification System (BCS), based on the aqueous solubility and intestinal permeability of a drug substance, has been widely used to predict the extent of drug absorption during the course of pharmaceutical development. Combined with product dissolution data, this system has gained a prominent role in regulatory process to determine if a drug formulated in an immediate release solid oral dosage form qualifies for waiver of in vivo bioequivalence studies. In parallel, the Biopharmaceutics Drug Disposition Classification System (BDDCS), using aqueous solubility and drug metabolism, takes on another venue to predict overall drug disposition. It has been suggested that the matrix of drug metabolism in BDDCS can be used to substantiate the classification of permeability by BCS. A total of 51 drugs were compiled in this study to examine the use of drug metabolism for predicting permeability. All compounds were classified as high permeability based on BCS, but only 73% of the compounds were found to exhibit extensive metabolism. Lipophilicity accounts for significant metabolism of many highly permeable drugs. Fourteen (14) out of 51 drugs have poor metabolism, suggesting that high permeability as defined by BCS does not necessarily dictate extensive metabolism. The drugs that have high permeability but poor metabolism are generally hydrophilic molecules with low molecular weight and are likely to be absorbed by active transport mechanisms. Based on the present data and literature information, it seems logical to predict that the extent of absorption is mostly complete (or ≥90%) if the drug is subject to a high degree of metabolism (e.g., ≥90%). The extent of drug metabolism may be useful in supporting permeability classification under certain circumstances.

Keywords: Biopharmaceutics Classification System; BCS; Biopharmaceutics Drug Disposition Classification System; BDDCS; drug permeability

Introduction

The extent of drug absorption and bioavailability are the central questions for pharmaceutical scientists during the course of drug discovery and development. In this context, the Biopharmaceutics Classification System (BCS)[1] has been widely used since 1995 and provided an invaluable tool for predicting how much a drug will be absorbed following the oral administration. The BCS categories drug substances into the following four classes based on their aqueous solubility and intestinal permeability:

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(1) Amidon, G. L.; Lennernäs, H.; Shah, V. P.; Crison, J. R. A theoretical basis for a biopharmaceutics drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* **1995**, *12*, 413–420.

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class 1: high solubility, high permeability

class 2: low solubility, high permeability

class 3: high solubility, low permeability

class 4: low solubility, low permeability

Over the years, the use of BCS in drug development has been expanded to regulatory practices in determining whether an in vivo bioequivalence study may be waived for immediate release solid oral dosage forms.[2] Specifically, according to a guidance issued by the U.S. Food and Drug Administration (FDA), waiver of in vivo bioequivalence studies can be granted for a highly soluble and highly permeable (i.e., BCS class 1) drug formulated in a rapid-dissolving drug product.[2] A drug substance is deemed highly soluble if the highest dose strength is soluble in 250 mL or less aqueous media over the pH range of 1−7.5. In the absence of evidence suggesting instability in the gastrointestinal tract, a drug substance is considered to be highly permeable when the extent of absorption in humans is 90% or more of an administered dose. This is based on extensive scientific research that revealed an excellent correlation between the human jejunal permeability measured using intestinal perfusion approaches and the fraction of dose absorbed obtained from pharmacokinetic studies in humans.[3] In view of these findings, the FDA guidance indicates that permeability classification can be determined directly by measuring the rate of mass transfer across human intestinal membrane or indirectly by estimating the extent of drug absorption in human pharmacokinetic studies.[2] Alternatively, nonhuman systems capable of predicting the extent of drug absorption in humans can be used (e.g., in vitro epithelial cell culture methods).[2]

Recently, a modified version of BCS termed Biopharmaceutics Drug Disposition Classification System (BDDCS) was proposed to better predict drug disposition.[4] The BDDCS was developed from the realization that the high permeability characteristics of BCS class 1 and class 2 drugs allow ready access to the metabolizing enzymes within hepatocytes, and thus there is also a good correlation between the extent of drug metabolism and measure of permeability defined under BCS.[4] In lieu of permeability, the BDDCS categorizes drug substances using the major route of drug elimination (or extent of drug metabolism) as follows:[4]

class 1: high solubility, extensive metabolism

class 2: low solubility, extensive metabolism

class 3: high solubility, poor metabolism

class 4: low solubility, poor metabolism

where the cutoff of "extensive metabolism" was originally set at ≥50% but later changed to ≥70% or 90% of an oral dose in humans.[4,5] Under the BDDCS criteria, 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.[4] The BDDCS is particularly useful in predicting the overall drug disposition, for example, the route of elimination, effects of transporters on oral absorption, direction of food effects, and significance of transporter-enzyme interplay in drug−drug interactions.[4]

It was suggested that BDDCS might provide an alternate (or additional) tool for characterizing intestinal permeability given the apparent correlation between the extent of drug metabolism and classification of drug permeability.[5] However, discrepancies between BCS and BDDCS have been observed where assignment of a drug class using BDDCS was not in accordance with that using BCS, and vice versa.[4−6] The questions that may arise are (1) to what extent drug metabolism can be used as a supporting evidence for intestinal permeability, and (2) under what circumstances drug metabolism may not be viable for permeability predictions. This paper is aimed to examine the use of drug metabolism for predicting intestinal permeability, and explore possible extension of waivers for in vivo bioequivalence testing using the matrix of drug metabolism.

Methods

A literature search was performed to compile a list of drug substances that had high intestinal permeability with no reported instability in the gastrointestinal tract.[2−28] Note that this list was assembled for illustration only and thus was

(2) *FDA Guidance for Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*; Food and Drug Administration: Rockville, MD, 2000. Retrieved from www.fda.gov/cder/guidance/index.htm.

(3) Lennernas, H. Human jejunal effective permeability and its correlation with preclinical drug absorption models. *J. Pharm. Pharmacol.* **1997**, *49* (7)), 627–638.

(4) Wu, C.-Y.; Benet, L. Z. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a Biopharmaceutics Drug Disposition Classification System. *Pharm. Res.* **2005**, *22*, 11–23.

(5) Benet, L. Z.; Amidon, G. L.; Barends, D. M.; Lennernas, H.; Polli, J. E.; Shah, V. P.; Stavchansky, S. A.; Yu, L. X. The use of BDDCS in classifying the permeability of marketed drugs. *Pharm. Res.* **2008**, *52*, 483–488.

(6) Takagi, T.; Ramachandran, C.; Bermejo, M.; Yamashita, S.; Yu, L. X.; Amidon, G. L. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain and Japan. *Mol. Pharmaceutics* **2006**, *3*, 631–643.

(7) Lennernas, H. Intestinal permeability and its relevance for absorption and elimination. *Xenobiotica* **2007**, *37*, 1015–1051.

(8) Martina, S. D.; Ismail, M. S.; Vesta, K. S. Cilomilast: Orally active selective phosphodiesterase-4 inhibitor for treatment of chronic obstructive pulmonary disease. *Ann. Pharmacother.* **2006**, *40*, 1822–1828.

(9) Ochs, H. R.; Otten, H.; Greenblatt, D. J.; Dengler, H. J. Diazepam absorption: effects of age, sex, and Billroth gastrectomy. *Dig. Dis. Sci.* **1982**, *27*, 225–230.

(10) Vaishali, P.; Stavchansky, S. Link between drug absorption, solubility and permeability measurements in Caco-2 cells. *J. Pharm. Sci.* **1998**, *87*, 1604–1607.

(11) Physicians' Desk Reference (PDR), 2008. P.O. Box 2244, Willison, VT, U.S.A.

not meant to be exhaustive. The drug substances collected were further examined with judicial assignment for "high permeability" classification based on the following methods:[2] (a) absolute bioavailability studies in humans where the oral bioavailability is determined using intravenous administration as a reference; (b) mass balance studies using unlabeled, stable isotopes, or a radiolabeled drug in humans; (c) urinary recovery of unchanged drug in humans; (d) in vivo intestinal perfusion studies in humans; or (e) in vitro permeation studies across a monolayer of cultured epithelial cells (e.g., Caco2 cells).

To facilitate comparisons between BCS and BDDCS, information on the degree of drug metabolism was obtained from standard references,[4,11,29−43] and the classification of "extensive metabolism" was given when ≥50% of the oral dose was metabolized in humans as applied in the original paper.[4] In addition, the estimated *n*-octanol/water partition coefficient for the unchanged form of a drug molecule

(12) Jung, D.; Mroszczak, E.; Bynum, L. Pharmacokinetics of ketorolac tromethamine in humans after intravenous, intramuscular and oral administration. *Eur. J. Clin. Pharmacol.* **1988**, *35*, 423–425.

(13) Yang, Y.; Faustino, P. J.; Volpe, D. A.; Ellison, C. D.; Lyon, R. C.; Yu, L. X. Biopharmaceutics classification of selected β-blockers: Solubility and permeability class membership. *Mol. Pharmaceutics* **2007**, *4*, 608–614.

(14) Urtasun, R. C.; Rabin, H. R.; Partington, J. Human pharmacokinetics and toxicity on high-dose metronidazole administered orally and intravenously. *Surgery* **1983**, *93* (1 Part 2), 145–148.

(15) Smith, P. F.; DiCenzo, R.; Morse, G. D. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. *Clin. Pharmacokinet.* **2001**, *40*, 893–905.

(16) Nelson, E.; Powell, J. R.; Conrad, K.; Likes, K.; Byers, J.; Baker, S.; Perrier, D. Phenobarbital pharmacokinetics and bioavailability in adults. *J. Clin. Pharmacol.* **1982**, *22* (2−3), 141–148.

(17) Mihaly, G. W.; Ward, S. A.; Edwards, G.; Nicholl, D. D.; Breckenridge, A. M. Pharmacokinetics of primaquine in man. I. Studies of the absolute bioavailability and effects of dose size. *Br. J. Clin. Pharmacol.* **1985**, *19*, 745–750.

(18) Zaccara, G.; Messori, A.; Moroni, F. Clinical pharmacokinetics of valproic acid. *Clin. Pharmacokinet.* **1988**, *15*, 367–389.

(19) Sumirtapura, Y. C.; Pamudji, J. S.; Ingriyani, I.; Sulistiyo, W.; Suhalim, H. Urinary excretion profiles of four oral cephalosporins in Indonesian healthy subjects. *Acta Pharm. Indones.* **2004**, *29*, 50−58.

(20) Shyu, W. C.; Shah, V. R.; Campbell, D. A.; Wilber, R. B.; Pittman, K. A.; Barhaiya, R. H. Oral absolute bioavailability and intravenous dose-proportionality of cefprozil in humans. *J. Clin. Pharmacol.* **1992**, *32*, 798–803.

(21) Schwinghammer, T. L.; Norden, C. W.; Gill, E. Pharmacokinetics of cephradine administered intravenously and orally to young and elderly subjects. *J. Clin. Pharmacol.* **1990**, *30*, 893–899.

(22) Debruyne, D.; Pyckelynck, J. P. Clinical pharmcokinetics of fluconazole. *Clin. Pharmacokinet.* **1993**, *24*, 10–27.

(23) Ramael, S.; Smedt, F. D.; Toublanc, N.; Otoul, C.; Boulanger, P.; Riethuisen, J.-M.; Stockis, A. Single-dose bioavailability of levetiracetam intravenous infusion relative to oral tablets and multiple-dose pharmacokinetics and tolerability of levetiracetam intravenous infusion with placebo in healthy subjects. *Clin. Ther.* **2006**, *28*, 734–744.

(24) Fish, D. N.; Chow, A. T. The clinical pharmacokinetics of levofloxacin. *Clin. Pharmacokinet.* **1997**, *32*, 101–119.

(25) Volpe, D. A. Permeability classification of representative fluoroquinolones by a cell culture method. *AAPS J.* **2004**, *6* (2), 1–6.

(26) Ward, M. E.; Musa, M. N.; Bailey, L. Clinical pharmacokinetics of lithium. *J. Clin. Pharmacol.* **1994**, *34*, 280–285.

(27) Brogden, R. N.; McTavish, D. Loracarbef. A review of its microbial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* **1993**, *45*, 716–736.

(28) Flor, S. Pharmacokinetics of ofloxacin: An overview. *Am. J. Med.* **1989**, *87* (6C), 24S–30S.

(29) Kivisto, K. T.; Kroemer, H. K. Use of probe drugs as predictors of drug metabolism in humans. *J. Clin. Pharmacol.* **1997**, *37* (Suppl), 40S–8.

(30) Tassaneeyakul, W.; Birkett, D. J.; McManus, M. E.; Tassaneeyakul, W.; Veronese, M. E.; Andersson, T.; Tukey, R. H.; Miners, J. O. Caffeine metabolism by human hepatic cytochromes P450: contributions of 1A2, 2E1 and 3A isoforms. *Biochem. Pharmacol.* **1994**, *47*, 1767–1776.

(31) Giembycz, M. A. Cilomilast: A second generation phosphodiesterase-4 inhibitor for asthma and chronic obstructive pulmonary disease. *Expert Opin. Invest. Drugs.* **2001**, *10*, 1361–1379.

(32) Leonard, R. G.; Talbert, R. L. Calcium-channel blocking agents. *Clin. Pharm.* **1982**, *1*, 17–33.

(33) Echizen, H.; Eichelbaum, M. Clinical pharmacokinetics of verapamil, nifedipine and diltiazem. *Clin. Pharmacokinet.* **1986**, *11*, 425–449.

(34) Tessari, p.; Pehling, G.; Nissen, S. L.; Gerich, J. E.; Service, F. J.; Rizza, R. A.; Haymond, M. W. Regulation of whole-body leucine metabolism with insulin during mixed-meal absorption in normal and diabetic humans. *Diabetes* **1988**, *37*, 512–519.

(35) Periclou, A.; Ventura, D.; Rao, N.; Abramowitz, W. Pharmacokinetic study of memantine in healthy and renally impaired subjects. *Clin. Pharmacol. Ther.* **2006**, *79*, 134–143.

(36) Lau, A. H.; Lam, N. P.; Piscitelli, S. C.; Wilkes, L.; Danziger, L. H. Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. *Clin. Pharmacokinet.* **1992**, *23*, 328–364.

(37) Evans, W. E., Schentag, J. J., Jusko, W. J., Eds. *Applied Pharmacokinetics*, 3rd ed.; Applied Therapeutics, Inc.: Vancouver, WA, 1992.

(38) McGourty, J. C.; Silas, J. H.; Fleming, J. J.; Mcburney, A.; Ward, J. W. Pharmacokinetics and beta-blocking effects of timolol in poor and extensive metabolizers of debrisoquin. *Clin. Pharmacol. Ther.* **1985**, *38*, 409–413.

(39) Brammer, K. W.; Farrow, P. R.; Faulkner, J. K. Pharmacokinetics and tissue penetration of fluconazole in humans. *Rev. Infect. Dis.* **1990**, *12*, S318–326.

(40) Benedetti, M. S.; Whomsley, R.; Nicolas, J.-M.; Young, C.; Baltes, E. Pharmacokinetics and metabolism of ^{14}C-levetiracetam, a new antiepileptic agent, in healthy volunteers. *Eur. J. Clin. Pharmacol.* **2003**, *59*, 621–630.

(41) Antonaccio, M. J.; Gomoll, A. Pharmacology, pharmacodynamics and pharmacokinetics of sotalol. *Am. J. Cardiol.* **1990**, *65*, 12A–21A; discussion 35A−36A.

(42) Carr, R. A.; Foster, R. T.; Lewanczuk, R. Z.; Hamilton, P. G. Pharmacokinetics of sotalol enantiomers in humans. *J. Clin. Pharmacol.* **1992**, *32*, 1105–1109.

(43) Funck-Brentano, C. Pharmacokinetic and pharmacodynamic profiles of d-sotalol and d,l-sotalol. *Eur Heart J.* **1993**, *4* (Suppl. H), 30–35.

(44) Kasim, N. A.; Whitehouse, M.; Ramachandran, C.; Bermejo, M.; Lennernas, H.; Husssain, A. S.; Junginger, H. E.; Stavchansky, S. A.; Midha, K. K.; Shah, V. P.; Amidon, G. L. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Mol. Pharmaceutics* **2004**, *1*, 85–96.

Table 1. Drugs with High Intestinal Permeability and Extensive Metabolism

	high permeability[a]		extensive metabolism[b]			
drugs	methods	refs	yes/no	refs	CLogP	remarks
antipyrine	d	2, 6, 7	yes	4, 29	0.20	
caffeine	d	2	yes	4, 30	−	
carbamazepin	d	2, 6, 7	yes	4, 11	2.38	
cilomilast	a	8	yes	31	−	
cyclosporine	d	7	yes	4	−	
desipramine	d	6, 7	yes	4	4.47	
diazepam	a	9	yes	4	3.17	
diltiazem	e	10	yes	4, 32, 33	2.70	
D-glucose	d	6	yes	4	−2.21	substrate of glucose transporters[6]
enalapril	d	6	yes	4	0.67	substrate of peptide transporters[6]
fluvastatin	d	2, 6	yes	11	4.05	
galantamine	a	11	yes	11	−	
isotretinoin	d	7	yes	11	−	
ketorolac	a	12	yes	4	−	
ketoprofen	d	2, 6, 7	yes	4	2.76	
labetalol	e	13	yes	4	−	
levodopa	d	6, 7	yes	4	−2.82	substrate of amino acid transporters[6]
L-leucine	d	6	yes	34	−1.67	substrate of amino acid transporters[6]
memantine	a	11	yes	11, 35	−	∼48% excreted unchanged in urine;partial hepatic metabolism[11]
metoprolol	d	2, 6, 7	yes	4	1.49	
metronidazole	a	14	yes	4, 36	−0.46	
naproxen	d	2, 6, 7, 10	yes	4, 11	2.82	
nevirapine	a	15	yes	4	−	
phenylalanine	d	6	yes	4	−1.56	substrate of amino acid transporters[6]
phenytoin	e	10	yes	4, 37	2.50	
phenobarbital	a	16	yes	4	1.37	
piroxicam	d	6, 7	yes	4	1.89	
primaquine	a	17	yes	4	−	
propranolol	d	2, 6, 7, 10	yes	4	2.75	
rosiglitazone	a	11	yes	4, 11	−	
salicylic acid	e	10	yes	4, 37	3.30	
theophylline	d	2	yes	4	−0.03	
tiagabine	a	11	yes	11	−	
timolol	e	13	yes	11, 38	−	
valproic acid	a	18	yes	4	2.76	
venlafaxine	b	11	yes	11	−	
verapamil	d	2, 6, 7	yes	4	4.47	

[a] Predicted or estimated by (a) absolute bioavailability studies, (b) mass balance studies, (c) amount of drug excreted unchanged in urine, (d) in vivo intestinal perfusion studies in humans, or (e) in vitro permeation studies using Caco2 cells. [b] Greater than or equal to 50% metabolism of an oral dose in humans.

(CLogP), where available from the literature, was recorded to assess compound lipophilicity. [6,44]

Results

As shown in Tables 1 and 2, a total of 51 compounds were classified with high intestinal permeability using the methods described in this paper. Overall, a high permeability classification was assigned to 20 (39%), 20 (39%), 6 (12%), and 8 (16%) compounds based on in vivo intestinal perfusion, absolute bioavailability, urinary recovery and in vitro permeation studies, respectively. Only one compound was classified as high permeability using mass balance data.

Of the 51 drugs with a "high permeability" classification, 37 (73%) drugs were found to undergo extensive metabolism

(45) Nozawa, T.; Toyobuku, H.; Kobayashi, D.; Kuruma, K.; Tsuji, A.; Tamai, I. Enhanced intestinal absorption of drugs by activation of peptide transporter PEPT1 using proton-releasing polymer. *J. Pharm. Sci.* **2003**, *92*, 2208–2216.

(46) Balimane, P. V.; Chong, S.; Patel, K.; Quan, Y.; Timoszyk, J.; Han, Y.-H.; Wang, B.; Vig, B.; Faria, T. N. Peptide transporter substrate identification during permeability screening in drug discovery: Comparison of transfected MDCK-hPepT1 Cells to Caco-2 cells. *Arch. Pharm. Res.* **2007**, *30*, 507–518.

(47) Maeda, T.; Takahashi, K.; Ohtsu, N.; Oguma, T.; Ohnishi, T.; Atsumi, R.; Tamai, I. Identification of influx transporter for the quinolone antibacterial agent levofloxacin. *Mol. Pharmaceutics* **2006**, *4*, 85–94.

Table 2. Drugs with High Intestinal Permeability and Poor Metabolism

drugs	high permeability[a] methods	high permeability[a] references	extensive metabolism[b]	urinary excretion[c] unchanged (%)	urinary excretion[c] refs	remarks
cefadroxil	c	19	no	>90%	19	substrate of peptide transporters[45,46]
cefprozil	a	20	no	60−70%	20	
cephalexin	c, d	6, 7, 19	no	>90%	19	substrate of peptide transporters[46]
cephradine	a	21	no	>80%	21	substrate of peptide transporters[45,46]
fluconazole	a	22	no	~80%	39	
levetiracetam	a	10, 23	no	~66%	11, 23, 40	hepatic P-450 not involved in metabolism[11]
levofloxacin	a, e	10, 24, 25	no	~87%	24	substrate of influx transporters[47]
lithium	c	26	no	100%	26	
loracarbef	c	27	no	>90%	27	substrate of peptide transporters[45,48]
ofloxacin	a, e	25, 28	no	>70%	28	substrate of multiresistance protein (MRP) 1[49]
pramipexole	a	11	no	90%	11	
pregabalin	c	11	no	~90%	11	substrate of amino acid transporters[11]
sotalol	a, e	13	no	>80%	41−43	absorbed via paracellular route[13,50]
varenicline	c	11	no	92%	11	substrate of renal organic cation transporters (OCTs)[51]

[a] Predicted or estimated by (a) absolute bioavailability studies, (b) mass balance studies, (c) amount of drug excreted unchanged in urine, (d) in vivo intestinal perfusion studies in humans, or (e) in vitro permeation studies using Caco2 cells. [b] Greater than or equal to 50% metabolism of an oral dose in humans. [c] Percentage of an oral dose recovered as unchanged drug in the urine.

(Table 1). The major route of metabolism for many of these drugs was through the liver and hepatic cytochrome P450 (CYP) isoenzymes were involved. However, it was noted that memantine, borderline high permeability, underwent partial metabolism in the liver and hepatic CYP enzyme system did not play a significant role in its metabolism.[11] Several of the compounds listed are substrates of CYP3A and UDP-glucuronosyltransferases (UGTs), and thus are also expected to exhibit significant first-pass metabolism in the intestinal mucosa.[4]

Table 2 displays 14 drugs falling in the category of "poor metabolism", which was evidenced by a large proportion (at least greater than 60%) of unchanged drug excreted in the urine. Most of these compounds had minimal or no metabolism except levetiracetam, an antiepileptic drug. Levetiracetam has an acetamide group attached to the compound that is subject to metabolism in vivo. About 24% of an oral dose of levetiracetam was reported to be metabolized through acetamide hydrolysis, which was, however, independent of hepatic CYP enzymes.[11]

Discussions

Drugs with High Permeability and Extensive Metabolism. The permeability of a drug can be affected by several factors, which may include physicochemical properties of the drug such as lipophilicity, molecular size, and hydrogen-bonding potential,[52] as well as physiological characteristics of the membrane such as passive diffusion versus active transport.[7] Drug lipophilicity has long been considered an important determinant of oral absorption because of the lipid nature of intestinal epithelial membrane.[7,52] Indeed, as shown in Table 1, many of the highly permeable compounds possess a fair degree of lipophilicity (as reflected by their high positive CLogP values), and thus are readily transported by passive transcellular diffusion across the intestinal membrane. However, there are exceptions to this general principle where a group of compounds that appear to be hydrophilic (ClogP < 0) are classified as "high permeability" based on the BCS criteria (Table 1). Some of these compounds, including D-glucose, levodopa, L-leucine, and phenylalanine, are known to be absorbed via the endogenous carrier-mediated mechanism.[6] Another possibility for transport of hydrophilic drugs is through intestinal paracellular route, which may be important for small molecules with molecular weight less than 250−300.[7] This is exemplified by the intestinal absorption of glucose at high concentrations where 'solvent drag' drives sugar molecules into paracellular space of the intestine.[53]

(48) Dantzig, A. H.; Duckworth, D. C.; Tabas, L. B. Transport mechanisms responsible for the absorption of loracarbef, cefixime, and cefuroxime axetil into human intestinal Caco-2 cells. *Biochim. Biophys. Biomembr.* **1994**, *1191*, 7–13.

(49) Terashi, K.; Oka, M.; Soda, H.; Fukuda, M.; Kawabata, S.; Nakatomi, K.; Shiozawa, K.; Nakamura, T.; Tsukamoto, K.; Noguchi, Y.; Suenaga, M.; Tei, C. Interactions of ofloxacin and erythromycin with the multidrug resistance protein (MRP) in MRP-overexpressing human leukemia cells. *Antimicrob. Agents Chemother.* **2000**, 1697–1700.

(50) Axel, A.; Potthast, H.; Moessinger, J.; Sickmuller, B.; Oeser, H. Biopharmaceutical characterization of sotalol-containing oral immediate release drug products. *Eur. J. Pharm. Biopharm.* **2004**, *58*, 145–150.

(51) Feng, B.; Obach, R. S.; Burstein, A. H.; Clark, D. J.; de Morris, S. M.; Faessel, H. M. Effect of human renal cationic transporter inhibition on the pharmacokinetics of varenicline, a new therapy for smoking cessation: A in vitro-in vivo study. *Clin. Pharmacol. Ther.* **2008**, *83*, 567–576.

(52) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235–249.

(53) Guyton, A. C.; Hall, J. E. *Textbook of Medical Physiology*, 9th ed.; W.B. Saunders Company: Philadelphia, PA, 1994.

It has been reported that the majority (~70%) of approved drugs are eliminated by metabolism due to their lipophilic nature.[54,55] Obviously, lipophilicity is not only critical in facilitating drug permeation into the intestinal membrane, but also important in allowing drug access to the metabolizing enzymes. Consistent with this notion is the current finding that all lipophilic drugs (with known positive ClogP values) undergo extensive metabolism. One question, however, can be raised for hydrophilic compounds (ClogP < 0) that are also found to be metabolized extensively (Table 1). This may be explained, in part, by the presence of functional groups on the substrate that is also an important characteristic of the compounds undergoing metabolism. A close examination of these hydrophilic compounds in Table 1 revealed that they all are small molecules and exhibit unique physicochemical properties. With the exception of metronidazole and theophylline that have borderline lipophilicity, most compounds are handled by the existing biological processes for endogenous chemicals or nutrients. For instance, there are two endogenous (aerobic and anaerobic) pathways involved in the degradation of glucose, which can occur in the tissues/cells wherever energy is needed.[53] Levodopa, an aromatic amino acid, can be metabolized to dopamine by a decarboxylase, which is part of the catecholamine biosynthesis pathway in vivo.[53] Likewise, both L-leucine and phenylalanine undergo biotransformation via the endogenous processes for amino acids.[53] It is interesting to note that all of the four compounds, albeit hydrophilic, are absorbed through the gastrointestinal mucosa by active transport and subject to enzymatic hydrolysis in the body.[53] Expression of such transporters and enzymes may be present in enterocytes as well as hepatocytes, and transporter-enzyme interplay may be important for the disposition of these compounds.[4]

As shown in Table 1, a number of drugs classified as high permeability undergo extensive metabolism. Hence, similar to previous reports,[4,5] the present study provides supportive evidence for the common characteristics of membrane permeability shared by the intestine and liver. It is further noted that the metabolism of most of these drugs takes place in the liver and/or intestinal mucosa. This is not surprising in view of the fact that the liver is generally considered the major site of drug metabolism, and the intestine is also known to make a significant contribution to first-pass elimination.[56] The intestinal first-pass metabolism can occur via a battery of enzymes, including phase I CYP3A enzymes and phase II conjugation enzymes (such as sulfotransferases and glucuronosyltransferases) that are located primarily in the mucosal enterocytes.[57] A similar process exists within hepatic parenchymal cells as drugs pass through the liver for the first time.[56] Note that there may be other metabolizing enzymes that are not localized in the liver or intestinal mucosa. For example, rivastigmine, a drug for the treatment of Alzheimer's disease, has been found to be completely hydrolyzed by cholinesterase at the site of action (i.e., central nervous system) after oral dosing, and hepatic enzymes are not involved in its metabolism.[11,58]

Drugs with High Permeability and Poor Metabolism. As listed in Table 2, 14 out of 51 drugs with high permeability under BCS were found to exhibit poor metabolism, indicating that a highly permeable drug may not be metabolized to a significant extent. This observation agrees with an earlier report[6] that correlations between BDDCS and BCS may have some limitations as BDDCS focuses on the liver and metabolizing enzymes located in hepatocytes whereas BCS refers to the intestine and drug permeation into enterocytes. Drug absorption process through the intestine may be different from its clearance kinetics in the liver. Intestinal absorption of drugs is known to comprise multiple parallel transport processes, including passive transcellular membrane diffusion, paracellular diffusion, and carrier-mediated transport.[7] In contrast, hepatic clearance of drugs may involve plasma protein binding, passive permeability, sinusoidal uptake, and cellular disposition.[59] It is suspected that there may be some distinct groups of compounds that can be absorbed through the intestinal membrane via one (or more) of the absorptive mechanisms, but have limited access to metabolizing enzymes due to their unique structures or physicochemical properties.

As displayed in Table 2, typical compounds categorized as high permeability under BCS, but having poor metabolism, include several beta-lactam antibiotics (cefadroxil, cefprozil, cephalexin, cephradine and loracarbef), antimicrobial quinolones (levofloxacin, ofloxacin), antiepileptics (levetiracetam, pregabalin), and other drugs for miscellaneous uses (e.g., pramipexole for Parkinson disease, and varenicline as an aid to smoking cessation treatment). Interestingly, many of these drugs act on the central nervous system. Almost all of the compounds are hydrophilic in nature, and some drugs are zwitterions (e.g., cephalosporins and quinolones) at the pH conditions in small intestine. Passive transcellular diffusion is well-known to constitute the main absorption process for drugs, even for fairly hydrophilic compounds such as atenolol and terbutaline.[7] However, a number of drugs are also absorbed through carrier-mediated transport.[4,7] For example, cephalosporins (cefadroxil, cephalexin, ceph-

(54) Van de Waterbeemd, H. The fundamental variables of the biopharmaceutics classification (BCS): A commentary. *Eur. J. Pharmacol. Sci.* **1998**, *7*, 1–3.

(55) Smith, D. A.; van de Waterbeemd, H. Pharmacokinetics and metabolism in early drug discovery. *Curr. Opin. Chem. Biol.* **1999**, *3*, 373–378.

(56) Thummel, K. E. Gut instincts: CYP3A4 and intestinal drug metabolism. *J. Clin. Invest.* **2007**, *117*, 3173–3176.

(57) Thummel, K. E.; Kunze, K. L.; Shen, D. D. Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv. Drug Delivery Rev.* **1997**, *27*, 99–127.

(58) Polinsky, R. J. Clinical pharmacology of rivastigmine: a new-generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Clin. Ther.* **1998**, *20*, 634–647.

(59) Baker, M.; Parton, T. Kinetic determination of hepatic clearance: Plasma protein binding and hepatic uptake. *Xenobiotica* **2007**, *37*, 1110–1134.

radine and loracarbef) have been shown to be the substrates of peptide transporters.[45,46,48] Organic anion transporting polypeptide (OATP) 1A2 was identified to be an influx transporter for quinolones such as levofloxacin.[47] As mentioned, absorption of drugs through paracellular diffusion has been considered as a possible intestinal transport route for small hydrophilic drug molecules.[7] Of the 14 drugs listed in Table 2, 4 compounds (levetiracetam, pramipexole, pregabalin, and varenicline) have MW < 250. Although information on the absorption mechanism of these 4 drugs is lacking, it has been demonstrated that sotalol (MW 308) was able to pass through the leaky intercellular junction via the paracellular pathway.[13,50]

The extent of drug metabolism can be determined by a number of factors related to the physicochemical properties of the substrate and the enzyme itself. Lack of lipophilicity may be a key that prevents the access of hydrophilic compounds to the metabolizing enzymes. In addition, the degree to which the shape of a compound complements that of the active site of an enzyme is important in determining whether it will be a substrate for the enzyme. For example, fluconazole is an azole compound with two five-membered nitrogen heterocyclic rings while quinolones (levofloxacine and ofloxacine) are pyridobenzoxazine derivatives. It has been reported that the presence of pyridobenzoxazine ring in ofloxacine decreases the parent drug metabolism.[11] Hence, the unique structures of these compounds may lend themselves steric hindrance to the binding of enzymes, thereby decreasing their potential for metabolism. In theory, however, for some lipophilic drugs with inherently high permeability, even if their affinity and accessibility to the enzymes are low, there is still possibility for them to be metabolized extensively (albeit slowly) as these drugs may be reabsorbed in the intestine and kidney due to their high permeability characteristics.

Classification of Intestinal Permeability by Drug Metabolism. It is clear that a number of highly permeable drugs classified under BCS are subject to significant metabolism based on the present study results. Nonetheless, the study also demonstrates that highly permeable drugs may be or may not be metabolized extensively. Using a cutoff of 50% oral dose, ~ 27% of "highly permeable" compounds were classified to exhibit poor metabolism. It could be envisioned that more compounds would fall in the category of "poor metabolism" should the cutoff be increased to 70% or 90%.

According to the FDA guidance, permeability classification under BCS can be made either by direct measurements of epithelial permeability across intestinal membrane or through indirect estimates of the extent of drug absorption from pharmacokinetic studies.[2] Retrospectively, the extensive scientific research conducted in 1990s has established the correlation between human jejunal permeability and extent of intestinal absorption.[3] Therefore, it is generally recognized that if a drug has ≥90% absorption, the drug must be highly permeable although actual permeability data may not be available. Based on actual permeability data from in vivo intestinal perfusion or in vitro Caco-2 cells permeation studies, 24 compounds would be included in Table 1 and 4 compounds in Table 2. In this way, ~86% of the compounds would be correctly predicted as high permeability by extensive metabolism (≥50%). Sotalol displayed low permeability in Caco-2 cells, but the extent of absorption was over 90%.[13] It has been pointed out that the low permeability through Caco-2 monolayers may be largely due to its low lipophiliity.[13] In addition, the difference between the tightness of the intercellular junction in vivo and in vitro may contribute to the disparity in the classification of permeability.[13] Hence, sotalol is classified as a high permeability drug.

The present investigation may not answer the question of whether a drug will possess the characteristics of high intestinal permeability inasmuch as the drug is metabolized extensively. It is suspected that the use of "extent of metabolism" for permeability prediction under BCS may be better suited for drug molecules with a fair degree of lipophilicity. A drug is likely to have high intestinal permeability if the drug is absorbed via passive transcelluar diffusion and metabolized extensively in the liver or intestinal mucosa. It seems uncommon that a drug exhibits both characteristics of low permeability and extensive metabolism. Yet, terbutaline is one example that has low permeability and is subject to extensive sulfate conjugation both in the liver and in the intestine.[7]

Based on this study and literature data, it may be logical to predict that if ≥90% of the administered dose is metabolized, the extent of drug absorption is mostly complete (or ≥90%). A question that has been posed for addition of drug metabolism to the current permeability criteria under BCS lies in the possible complication of enzyme polymorphism. For instance, using 70% as the cutoff for extent of metabolism, in the event that a drug is metabolized to >70% by a polymorphic enzyme (e.g., CYP 2D6) in extensive metabolizers, but to a lesser amount (<70%) in poor metabolizers, a difference in permeability classification will result between the two populations.

(60) Blume, H. H.; Schug, B. S. The biopharmaceutics classification system (BCS): Class III drugs - better candidates for BA/BE waiver. *Eur. J. Pharm. Sci.* **1999**, *9*, 117–121.

(61) Yu, L. X.; Amidon, G. L.; Poli, J. E.; Zhao, H.; Mehta, M. U.; Conner, D. P.; Shah, V. P.; Lesko, L. J.; Chen, M.-L.; Lee, V. H. L.; Hussain, A. S. Biopharmaceutics classification system: The scientific basis for biowaiver extensions. *Pharm. Res.* **2002**, *7*, 921–925.

(62) Linderberg, M.; Kopp, S.; Dressman, J. B. Classification of orally administered drugs on the World Health Organization Model List of Essential Medicines according to the biopharmaceutics classification system. *Eur. J. Pharm. Biopharm.* **2004**, *58*, 265–278.

(63) Poli, J. E.; Yu, L. X.; Cook, J.; Amidon, G. L.; Borchardt, R. T.; Burnside, B. A.; Burton, P. S.; Chen, M.-L.; Conner, D. P.; Faustino, J.; Hawi, A. A.; Hussain, A. S.; Joshi, H. N.; Kwei, G.; Lee, V. H. L.; Lesko, L. J.; Lipper, R. A.; Loper, A. E.; Nerukar, S. G.; Polli, J. W.; Sanvordeker, D. R.; Taneja, R.; Upporr, R. S.; Vattikonda, C. S.; Wilding, I.; Zhang, G. Summary workshop report: Biopharmaceutics classification system - Implementation challenges and extension opportunities. *J. Pharm. Sci.* **2004**, *93*, 1375–1381.

Since the introduction of the FDA guidance on BCS, the possible extension of waiver for in vivo bioequivalence studies based on BCS has been the subject of extensive research and discussions.[60−67] The development of BDDCS may offer an opportunity to expand the role of BCS in regulatory applications by using "extent of metabolism" to classify the intestinal permeability of drugs. Future work is encouraged in the field to facilitate the extension of biowaivers to more drug products.

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(64) Yazdanian, M.; Briggs, K.; Jankovski, C.; Hawi, A. The 'high solubility' definition of the current FDA guidance on biopharmaceutics classification system may be too strict for acidic drugs. *Pharm. Res.* **2004**, *21*, 293–299.

(65) Rinaki, E.; Dokoumetzidis, A.; Valsami, G.; Macheras, P. Identification of biowaivers among Class II drugs: Theoretical justification and practical examples. *Pharm. Res.* **2004**, *21*, 1567–1572.

(66) Lennernas, H.; Abrahamsson, B. The use of biopharmaceutic classification of drugs in drug discovery and development: Current status and future extension. *J. Pharm. Pharmacol.* **2005**, *57*, 273–285.

(67) Fagerholm, U. Evaluation and suggested improvements of the biopharmaceutics classification system (BCS). *J. Pharm. Pharmacol.* **2007**, *59*, 751–757.