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Functional Role of P-Glycoprotein in Limiting Intestinal Absorption of Drugs: Contribution of Passive Permeability to P-Glycoprotein Mediated Efflux Transport

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Abstract: The aim of the present study is to evaluate the quantitative contribution of passive permeability to P-glycoprotein-mediated (P-gp-mediated) efflux and the functional activity of P-gp in determining intestinal absorption of drugs, and demonstrate the relationship between efflux parameters and intestinal permeability. MDRI-MDCKII cell monolayer permeability, human intestinal absorption (HIA), and solubility data were systematically collected from the literature. Drugs were classified as a total of 63 P-gp substrates (P-gpS) and 73 nonsubstrates (NS) on the basis of efflux ratio or calcein AM inhibition and ATPase activity assays. Efflux parameters, efflux ratio (ER) and absorption quotient (AQ), were correlated to the monolayer permeability. MDRI-MDCKII cell monolayer permeability characteristics were found to be distinctly different between P-gpS and NS datasets. The ER for P-gpS was found to increase with absorptive permeability until 20 $nm \cdot s^{-1}$, but reduced for P-gpS with high absorptive permeability. The AQ showed a linear inverse relationship with absorptive permeability. Overall, efflux parameters, ER and AQ, indicated that the transport of P-gpS with moderate passive permeability is highly attenuated by P-gp, while passive permeability overrules the P-gp-mediated efflux for high-permeability molecules. Most of the P-gpS were found towards the upper limits of molecular weight (>500) and calculated total polar surface area (>75 $Å^2$). This dataset indicated that unfavorable chemical features of P-gpS limit passive permeability and thus are more susceptible to P-gp-mediated efflux. In conclusion, passive permeability versus P-gp-mediated efflux determines intestinal permeability of P-gpS, where P-gp limits absorption of only moderately permeable compounds. Thus, integrating these factors with drug characteristics of the Biopharmaceutics Classification System (BCS) class better predicts the functional role of P-gp in limiting intestinal drug absorption.

Keywords: P-glycoprotein; intestinal permeability; Biopharmaceutics Classification System; absorption quotient; Lipinski's rule-of-5

Introduction

Absorption of drugs from the gastrointestinal (GI) tract is very complex and is influenced by many factors, which fall into three classes. The first class of factors comprises physicochemical properties of drugs, including pK_a, solubility, stability, diffusivity, and lipophilicity. The second class comprises physiological factors such as GI pH, gastric emptying, intestinal transit, gut wall metabolism, and active transport including drug efflux. Finally, the third class includes the formulation factors such as surface area, drug particle size and crystal form, and type of dosage form. Mathematical analysis of kinetics and dynamics of these processes in the GI tract indicated solubility and permeability

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as the fundamental properties controlling oral drug absorption. On the basis of these two fundamental processes, Amidon and co-workers proposed the Biopharmaceutics Classification System (BCS),[1] which currently serves as regulatory and industrial guidelines. The objective of the BCS is to predict in vivo performance of drug products from in vitro measurements of solubility and permeability.

Permeability is an important, but still unpredictable, determinant of absorption, and it is informative to explore mechanisms contributing to permeability given the interest in development of structure-based computational models of this property. More recently, the role of efflux transporters in determining the permeability and overall bioavailability of drugs has gained considerable attention.[2] P-gp, an energy-dependent transmembrane drug efflux pump, is localized in a wide range of tissues including enterocytes of the GI tract.[3] An increasing number of drugs, including HIV protease inhibitors such as indinavir, ritonivir, and saquinavir and anticancer drugs such as paclitaxel, docetaxel, etc. have been reported to be substrates for P-gp. In vivo studies confirmed that P-gp significantly limits the oral bioavailability of several drugs, where intestinal permeability showed dose dependence with increased permeability as lumen concentration increases.[4] At the same time, the literature also indicated no influence of P-gp on bioavailability for a number of drugs.[5,6] P-gpS-like etoposide, indinavir, ritonavir, saquinavir, and verapamil, which exhibited varying degrees of efflux, showed dose-independent in vivo kinetics in absorption (C_{max} and T_{max}) and bioavailability (AUC).[6] On the contrary, P-gpS-like digoxin,[7] paclitaxel,[8] talinolol,[9,10] and saquinavir,[11] which exhibited high efflux, showed improved bioavailability in the presence of P-gp inhibitors. It should be appreciated that both passive permeability and the P-gp efflux process, operating in mutually opposite directions, contribute to overall drug permeability and thus influence the bioavailability. A number of P-gp substrates (P-gpS) are practically not polarized despite drug efflux, which can be ascribed to a lower control by P-gp caused by higher passive transmembrane movement rate and/or to per se a lower activity of P-gp. The permeability of P-gpS is the net result of the passive influx rate minus the P-gp-mediated active efflux rate. A quantitative relationship between P-gp activity and intestinal permeability helps in screening of drug candidates for P-gp-mediated efflux, and predicting the permeability limitation during the early phase of development helps in either early elimination of the drugs from development or providing an opportunity to handle them on the basis of drug delivery strategies.

Therefore, the objectives of the present study were to assess the quantitative contribution of P-gp-mediated efflux in limiting oral bioavailability of drugs and to explore the possibilities to predict the intestinal absorption of drugs from in vitro permeability studies and physicochemical properties. We also attempted to correlate the transport processes and human intestinal absorption (HIA) of P-gpS and non-substrates (NS), on the basis of the BCS.

Methods

Permeability and HIA Data. All the permeability data was collected from two independent studies from Preclinical Drug Metabolism and Pharmacokinetics, GlaxoSmith-Kline.[12,13] Monolayer efflux studies in these experiments were carried out using multidrug resistance transfected MDCK type II (MDRI-MDCKII) cell lines. To avoid any bias, all

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the molecules in both studies were considered, and mean value with minimum SD was selected in cases where same compound was reported in both of the studies. Compounds were classified on the basis of the efflux ratio (ER, $P_{app,BA}/P_{app,AB} > 1.5$) from bidirectional transport studies. However, drugs with no efflux but positive for calcein AM inhibition and ATPase assays were considered as P-gp substrates.

HIA data was taken from previous compilations and standard references.[14−17] In order to retrieve maximum HIA data for the drug listed, an extensive Medline database search was also performed using the keywords or phrases "absolute bioavailability", "drug", and "oral absorption" (http://www.pubmed.com). The mean of the range was considered when the range was given. Values which were referred a greater number of times were considered, in the case of different reported values. However, HIA values for some of the compounds listed were not available from the literature.

Solubility, Maximum Dose Strength, and Dose Number (Do). The solubility of the compounds was obtained from standard references.[15,18,19] For a more conservative estimate of solubility, the lower limit of the range defined in the USP was considered, when specific values of solubility were not available.[18]

Maximum dose strength was primarily obtained from the orange book (online version: http://www.fda.gov/cder/ob/default.htm) and USP DI.[15] However, a few drugs in the dataset are not available as oral dosage forms, and the Do was not calculated for these drugs. The following equation was used to calculate the Do:

$$\mathrm{Do} = \frac{\mathrm{Mo}}{(\mathrm{Vo})(\mathrm{Cs})} \quad (1)$$

where Mo is the highest dose strength (mg), Cs is the solubility (mg/mL), and Vo is 250 mL, the minimum volume that is available for a formulation to disintegrate and dissolve.

Lipinski's Rule-of-5: Physicochemical Properties. *Clog P* was calculated with ChemDraw Ultra 6.0 (Cambridge Soft. Corp., Cambridge, MA) using chemical structure inputs. Total polar surface area (TPSA), captured as the van der Waals surface area of all nitrogen and oxygen atoms plus their attached hydrogen atoms, was taken as an indicator for number of hydrogen-bonding donors and acceptors. TPSA was calculated with a Web-based molecular descriptor calculator (http://www.molinspiration.com) using SMILES notations or chemical structure inputs. SMILES notations were obtained from World Drug Index demo version 2.0. Chemical structures of drugs as depicted in *The Merck Index* were drawn. Molecular weight (MW) was taken from *The Merck Index*.[19]

Statistics. A nonparametric Mann−Whitney rank sum test was used to assess the statistical significance between permeability and physicochemical characteristics of P-gpS and NS datasets, at a significance level of $p < 0.001$ (SigmaStat 2.03, SPSS Inc., IL).

Results and Discussion

MDRI-MDCKII Monolayer Permeability of P-gpS and NS. The mean apparent permeability in absorptive direction ($P_{app,AB}$) is 430.8 nm·s^{-1} and 150.3 nm·s^{-1} for 73 passively permeating NS and 63 P-gpS, respectively. A significant difference was found between the mean permeability of the two datasets ($p < 0.001$). It is interesting to find that 64 out of 73 (∼88%) NS showed $P_{app,AB}$ of more than 100 nm·s^{-1}, while 38 out of 63 (∼60%) P-gpS have permeability less than 100 nm·s^{-1} (Figure 1). This dataset indicates that P-gpS have less permeability than NS, which could be due to (i) unfavorable physicochemical properties and/or (ii) P-gp efflux significantly affecting $P_{app,AB}$ (Tables 1 and 2). MDCK cells, a dog renal epithelial cell line the cells of which differentiate into columnar epithelium in a shorter period of time than Caco-2 cells (3 days vs 21 days for Caco-2 cells), showed a good correlation with permeation of passively absorbed drugs in Caco-2 monolayers, a well-established model for screening intestinal permeability of drugs with in vivo HIA.[20] MDCK cells transfected with human MDRI expressing human P-gp have been used as model for the intestinal mucosa.[21−23] Similar to the MDRI expressed in Caco-2 cells, the human P-gp in MDRI-MDCKII cells is located on the apical side of polarized cell monolayers, leading to efflux of P-gp substrates.

The mean secretory permeability of P-gpS was high ($P_{app,BA}$ = 395.2 nm·s^{-1}), with 50 out of 63 (∼79%)

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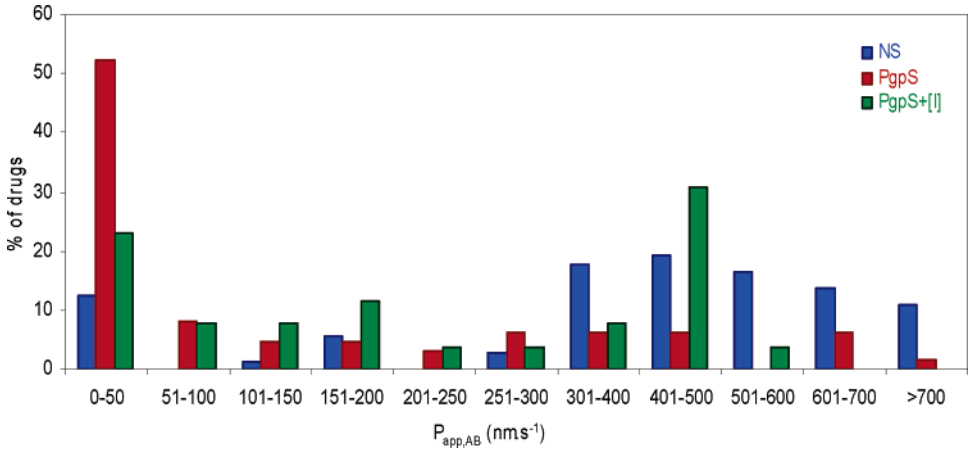

Figure 1. Distribution of MDRI-MDCKII monolayer absorptive permeability ($P_{app,AB}$) of NS and P-gpS in the absence and presence of specific P-gp inhibitor GF120918. Percentage of drugs was indicated for 73 NS and 63 P-gpS and with available data of permeability in the presence of inhibitor (26 P-gpS).

molecules having permeability of more than 100 nm·s^{-1}. The mean ER of P-gpS was found to be 16.6, while inhibition of P-gp with 2 μM GF120918 reduced the ER of P-gpS to near unity (Table 2). The mean permeability of P-gpS in the presence of inhibitor, indicative of passive permeability ($P_{PD,AB}$), was found to be 244.0 nm·s^{-1} (for 26 drugs). Further, drugs which showed no efflux but activated P-gp ATPase and inhibited calcein AM uptake were found to be highly permeable (e.g., chlorpromazine, ketoconazole, mebendazole, midazolam, nicardipine, nifedipine, nitrendipine). Interestingly, $P_{PD,AB}$ of P-gpS was significantly less ($p < 0.001$) than the passive permeability of NS. These results collectively suggest that most of the P-gpS have limited permeability attributable to P-gp-mediated efflux; however, P-gpS also shows less intrinsic passive transport.

Relationship between Efflux Parameters and MDRI-MDCKII Monolayer Permeability. The ER of P-gpS, indicative of the functional activity of P-gp-mediated drug efflux, was found to be in the range of 1.5−261.5. Figure 2 shows the relationship between $P_{app,AB}$ and ER. An interesting observation is that the ER increased as the $P_{app,AB}$ increased from 1 to 20 nm·s^{-1} but reduced with further increase in $P_{app,AB}$. This relationship indicates that moderate passive permeability is necessary for P-gp to demonstrate significant drug efflux.[24] The P-gp effect on the intestinal permeability or the rate and extent of GI absorption of drugs with high passive permeability was observed to be minimal. P-gpS with permeability > 100 nm·s^{-1} showed a mean ER of only 2.04, while low-permeable P-gpS (<100 nm·s^{-1}) have a mean ER of 26.37 ($p < 0.001$). For the drugs with high permeability, the secreted molecules could be rapidly reabsorbed back into the enterocytes, and the absorption barrier effect of P-gp would then become insignificant. The other hypothesis could be saturation of P-gp activity transport. It is obvious from Figure 2 that there are two sets of drugs deviating from this concept. Drugs such as saquinavir, paclitaxel, and actinomycin, which are reported to be substrates with high affinity, showed high ER values with $P_{app,AB}$ of less than 2 nm·s^{-1}. For these drugs such a low $P_{app,AB}$ is mainly due to transport attenuation by P-gp. The other drugs that are exceptions to the trend include neostigmine, puromycin, etopside, acrivastin, cyclosporine, and colchicines, which showed low ER even though they exhibited moderate permeability. Availability of the molecules for P-gp is not the only requirement for drug efflux, but the affinity of the molecules to P-gp also plays important role, and the exceptions may be attributed to the affinity for P-gp.[25]

Further to quantify and express to what extent P-gp-mediated efflux activity affects substrate transport across polarized epithelium, we calculated the absorptive quotient (AQ) and secretory quotient (SQ) for P-gpS with the available data of inhibition studies with 2 μM GF120918.[26]

$$\mathrm{AQ} = \frac{P_{\mathrm{PD,AB}} - P_{\mathrm{app,AB}}}{P_{\mathrm{PD,AB}}} = \frac{P_{\mathrm{P-gp,AB}}}{P_{\mathrm{PD,AB}}} \qquad (2)$$

$$\mathrm{SQ} = \frac{P_{\mathrm{app,BA}} - P_{\mathrm{PD,BA}}}{P_{PD,BA}} = \frac{P_{P-gp,BA}}{P_{\mathrm{PD,BA}}} \qquad (3)$$

where $P_{\mathrm{P-gp,AB}}$ and $P_{\mathrm{P-gp,BA}}$ express the effect P-gp would have in attenuating absorption transport (AQ) and enhancing secretory transport (SQ) of its substrates, respectively; and $P_{\mathrm{PD,AB}}$ and $P_{\mathrm{PD,BA}}$ are passive permeability in the absorption and secretory directions. AQ quantifies the functional activity of P-gp in absorption transport and lies between 0 and 1.

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Table 1. Summary of Literature and Calculated Data of MDRI-MDCKII Permeability, Physicochemical Properties, Dose Number, and the Percentage of Oral Dose Absorbed in Humans for 73 Drugs Which Are Not Substrates to P-gp (NS)[a]

no.	nonsubstrates	$P_{app,AB}$[b] ($nm \cdot s^{-1}$)	$P_{app,BA}$[b] ($nm \cdot s^{-1}$)	ER	MW	TPSA	*C* log *P*	Do[c]	% HIA[d]	BCS class[f]
1	alprenolol	461.0	467.0	1.0	249.0	41.5	2.65	na[g]	93	
2	amantadine	427.0	405.0	0.9	151.3	26.0	2.00	0.0010	95	I
3	amitriptylline HCl	474.0	636.0	1.3	277.3	3.2	4.85	0.0040	95	I
4	antipyrine	792.0	742.0	0.9	188.0	26.9	0.20	na	97	
5	atenolol	2.6	3.2	1.2	266.3	84.6	−0.11	0.0151	50	III
6	biperidine	515.0	491.0	1.0	311.5	23.5	4.42	0.0080	100	I
7	bromocriptine	194.0	245.0	1.3	654.6	118.2	6.27	0.0500	30	I
8	bufuralol	641.0	500.0	0.8	261.4	45.4	3.40	na	na	
9	buspirone HCl	547.0	517.0	0.9	422.0	69.6	1.22	0.0012	87	I
10	carbamazepine	602.0	592.0	1.0	236.3	48.0	1.98	80.0000	70	II
11	chlorpheniramine	468.0	436.0	0.9	274.8	16.1	3.15	0.0001	34	I
12	chlorprothixene	260.0	328.0	1.3	315.9	3.2	4.41	20.0000	na	II
13	clemastine funurate	331.0	429.0	1.3	343.9	12.5	5.55	0.1072	90	I
14	clomipramine	369.0	526.0	1.4	314.9	6.5	5.92	0.0160	80	I
15	clonidine	529.0	522.0	1.0	230.1	36.4	1.43	0.0000	100	I
16	cyclobenzaprine	385.0	375.0	1.0	275.4	3.2	4.70	0.0004	100	I
17	desipramine	551.0	568.0	1.0	266.4	15.3	4.47	0.0120	100	I
18	diphenhydramine	698.0	637.0	0.9	255.4	12.5	3.54	0.0002	50	I
19	doxapram	451.0	635.0	1.4	378.5	32.8	3.24	na	60	
20	doxepin	542.0	622.0	1.1	279.4	12.5	4.09	0.0040	27	I
21	doxorubicin	14.5	9.7	0.7	543.5	206.1	−1.45	na	12	
22	doxylamine	359.0	302.0	0.8	270.4	25.4	2.44	na	na	
23	flumazenil	597.0	552.0	0.9	303.3	64.4	1.09	na	95	
24	fluoxetine	521.0	617.0	1.2	309.3	21.3	4.57	0.0048	95	I
25	flurazepam	705.0	621.0	0.9	387.9	35.9	4.42	0.0002	100	I
26	fluvoxamine	317.0	380.0	1.2	318.3	56.9	4.99	0.0121	53	I
27	guanbenz	598.0	543.0	0.9	231.1	74.3	2.98	0.0160	79	I
28	guanfacine	130.0	159.0	1.2	246.1	79.0	1.37	0.0002	80	I
29	haloperidol	556.0	579.0	1.0	375.9	40.5	3.85	2.0000	100	II
30	imipramine	393.0	414.0	1.1	280.4	6.5	5.04	0.0020	100	I
31	indomethacine	616.0	597.0	1.0	357.8	68.5	4.18	30.0000	100	II
32	itraconazole	571.0	552.0	1.0	705.6	104.7	6.53	400.0000	85	II
33	ketamine	748.0	695.0	0.9	237.7	29.1	2.93	na	na	
34	lidocaine	842.0	825.0	1.0	234.3	32.3	1.95	na	35	
35	lorcainide	448.0	648.0	1.4	370.9	23.5	4.62	na	na	
36	mannitol	8.3	7.3	0.9	182.2	121.4	−4.67	0.0120	15	III
37	maprotiline	455.0	480.0	1.1	277.4	12.0	4.39	0.2100	95	I
38	mephentermine	537.0	468.0	0.9	163.3	12.0	2.29	na	na	
39	meprobamate	344.0	334.0	1.0	218.3	104.7	0.92	1.6000	90	*h*
40	metergoline	179.0	216.0	1.2	403.5	46.5	5.95	na	na	
41	methotrexate	10.5	7.1	0.7	454.5	210.5	−0.05	1.0000	65	III
42	metoprolol	296.0	359.0	1.2	267.4	50.7	1.35	0.0004	98	I
43	mexilitene	682.0	593.0	0.9	179.3	35.3	2.57	0.0020	100	I
44	naloxone	495.0	638.0	1.3	327.4	70.0	0.16	na	95	
45	naltrexone	402.0	418.0	1.0	341.4	70.0	1.42	0.0020	100	I
46	nitrazepam	389.0	456.0	1.2	281.3	87.3	2.32	2.0000	78	*h*
47	nordazepam	647.0	602.0	0.9	270.7	41.5	3.02	na	na	
48	nortriptyline	337.0	468.0	1.4	263.4	12.0	4.32	0.0100	100	I
49	noscapine	642.0	661.0	1.0	413.4	75.7	3.02	na	100	
50	oxprenolol	308.0	420.0	1.4	265.4	50.7	1.69	0.0032	95	I
51	perphenazine	305.0	449.0	1.5	286.0	31.6	2.86	6.4000	70	II
52	pheniramine	308.0	434.0	1.4	240.3	16.1	2.44	na	77	
53	practolol	12.4	16.4	1.3	266.4	70.6	0.75	na	100	
54	procyclidine	703.0	665.0	0.9	287.4	23.5	4.59	0.0006	100	I
55	progabide	684.0	601.0	0.9	334.8	75.7	2.90	na	60	
56	promazine	336.0	388.0	1.2	284.4	8.2	4.20	0.0008	40	I
57	promethazine	430.0	548.0	1.3	284.4	8.2	4.26	0.0001	25	I
58	propranolol	496.0	514.0	1.0	295.0	41.5	2.75	0.0048	90	I
59	pyridostigmine	10.2	12.6	1.2	181.2	33.4	−4.51	0.0024	10	III
60	ranitidine	14.4	19.5	1.4	314.4	86.3	0.67	0.0012	52	III
61	scopolamine	171.0	194.0	1.1	303.4	62.3	0.29	0.0002	95	I
62	selegiline	703.0	535.0	0.8	187.3	3.2	3.02	0.0002	100	I
63	sulfasalazine	6.2	10.3	1.7	398.4	141.3	3.88	200.0	59	IV
64	sumatriptan	9.5	13.0	1.4	295.4	65.2	0.58	0.0040	14	III
65	tacrine	438.0	409.0	0.9	198.3	38.9	3.27	0.0048	17	I
66	toxindole	519.0	598.0	1.2				na	na	
67	trazodone	747.0	698.0	0.9	371.9	45.8	3.17	0.4000	90	I
68	triamterene	185.0	184.0	1.0	253.3	129.6	1.61	40.0000	54	II
69	trimipramine	405.0	372.0	0.9	294.4	6.5	5.44	4.0000	100	II
70	warfarin	781.0	646.0	0.8	308.3	67.5	2.90	0.0000	98	I
71	yohimbine	429.0	501.0	1.2	354.5	65.5	2.17	na	na	
72	zimeldine	676.0	685.0	1.0	317.2	16.1	3.19	na	na	
73	zolpidem	694.0	788.0	1.1	307.4	37.6	2.83	0.0017	100	I

[a] Drug was considered as nonsubstrate (NS) when it showed ER < 1.5 and negative to calcien AM and ATPase assays. [b] MDRI-MDCKII monolayer bidirectional permeability as reported.[12,13] [c] Calculated by eq 1 using solubility data from the literature.[15,18,19] [d] Mean values of % HIA obtained from individual drug references or from standard compilations.[14−17] [f] Solubility criteria for the BCS was based on Do with a cutoff of Do ≤ 1 for high solubility and Do ≥ 2 for low solubility. The permeability criterion from monolayer transport was set as $P_{app,AB} \leq 20$ $nm \cdot s^{-1}$ for low permeability and $P_{app,AB} \geq 100$ $nm \cdot s^{-1}$ for high permeability. [g] Not available. [h] Borderline class.

Table 2. Summary of Literature and Calculated Data of MDRI-MDCKII Permeability, Physicochemical Properties, Dose Number, and the Percentage of Oral Dose Absorbed in Humans for 63 P-gp Substrates[a]

no.	P-gpS	$P_{app,AB}$[b] (nm·s^{-1})	$P_{app,BA}$[b] (nm·s^{-1})	ER	$P_{PD,AB}$[c] (nm·s^{-1})	MW	TPSA	*C* log *P*	Do[d]	% HIA[e]	BCS class[f]
74	acrivastine	11.7	43.4	3.7	16.2	348.4	53.4	1.13	na[g]	88	
75	actinomycin	0.2	16.0	76.2		1255.4	103.3		na	5	
76	amprenavir	21.7	703.0	32.4	401.0	505.6	131.2	3.29	3.1579	70	*h*
77	astemizole	184.0	408.0	2.2	461.0	458.6	42.3	5.94	4.0000	90	II
78	BW 1019W91	242.0	598.0	2.5							
79	BW 1136U89	355.0	434.0	1.2							
80	BW 1288U89	216.0	627.0	2.9							
81	BW 1351W91	268.0	408.0	1.5							
82	BW 1379W91	157.0	778.0	5.0							
83	BW 565C81	405.0	504.0	1.2							
84	cetirizine	25.4	217.0	8.5	40.0	388.9	53.0	2.08	0.0012	60	*h*
85	chloroquine	44.9	172.0	3.8		319.9	28.2	5.06	0.0060	100	*h*
86	chlorpromazine	438.0	477.0	1.1		318.9	8.2	5.30	0.0004	96	I
87	cimetidine	4.0	18.9	4.8	5.2	252.8	88.9	0.38	0.8000	64	III
88	claritromycin	10.8	336.0	31.1		748.0	173.7	2.09	200.000	100	IV
89	colchicine	17.9	202.0	11.3		399.4	94.1	1.19	0.0001	44	III
90	cyclosporin A	15.9	153.0	9.6		1206.0	253.4	3.29	4.0000	40	IV
91	daunorubicin	5.5	77.7	14.2		527.5	185.9	0.06	na	10	
92	dexamethasone	43.5	537.0	12.3		392.5	100.9	1.78	0.0200	81	*h*
93	diltiazem	413.0	676.0	1.6	465.0	414.5	59.1	3.64	0.0048	90	I
94	dipyridamole	28.5	646.0	22.7		504.6	145.4	2.53	0.0120	60	*h*
95	domperidone	18.5	577.0	31.2	157.0	425.9	78.8	4.27	2.0000	93	IV
96	eletriptan	14.8	663.0	44.8	429.0	382.5	53.2	3.35	16.0000	50	IV
97	emetine	9.6	281.0	29.2		480.6	52.2	4.95	0.4000	na	III
98	erythromycin	0.9	13.5	14.4		519.7	116.2	1.61	10.0000	35	IV
99	etoposide	21.4	60.6	2.8		588.6	160.9	−1.89	4.0000	50	*h*
100	famciclovir	71.2	226.0	3.2	119.0	321.3	122.2	0.08	0.2000	60	*h*
101	Hoechst 33342	3.4	26.0	7.8					na	na	
102	indinavir	8.7	176.0	20.3	85.0	613.8	118.0	3.68	0.0016	62.5	III
103	ketoconazole	316.0	323.0	1.0		531.4	69.1	3.64	80.0000	76	II
104	labetolol	40.9	362.0	8.9	73.7	328.4	95.6	2.50	0.0242	14	*h*
105	levomeprazine	347.0	535.0	1.5	478.0	328.5	17.4	5.33	na	21	
106	loperamide	77.7	773.0	9.9	456.0	477.5	43.8	4.66	0.1000	40	*h*
107	loratadine	264.0	502.0	1.9		382.9	42.4	5.05	na	90	
108	mebendazole	714.0	648.0	0.9		295.3	84.0	3.08	200.000	5	II
109	mequitazine	260.0	731.0	2.8	462.0	322.5	8.2	5.21	na	na	
110	methysergide	174.0	753.0	4.3	394.0	353.5	57.5	2.22	0.0008	13	I
111	midazolam	609.0	613.0	1.0		325.8	30.2	3.22	0.0012	36	I
112	mitoxantrone	1.9	6.4	3.4		444.5	163.2	0.24	na	85	
113	monensin	102.0	294.0	2.9		670.9	173.6	3.00	na	na	
114	nalbuphine	140.0	303.0	2.2	156.0	373.5	73.2	1.39	na	12	
115	nelfinavir	35.3	786.0	22.3	197.0	567.8	101.9	5.53	0.0758	78	*h*
116	neostigmine	6.9	15.4	2.2	8.5	223.3	29.5	−2.81	na	10	
117	nicardipine	614.0	661.0	1.1		479.5	113.7	5.52	0.0040	95	I
118	nifedipine	610.0	765.0	1.3		346.3		3.41	4.0000	45	II
119	nitrendipine	604.0	483.0	0.8		360.4	110.5	4.02	na	15	
120	pirenzapine	1.8	6.4	3.6	3.2	351.4	74.2	0.16	0.0000	25	III
121	prazosin	143.0	661.0	4.6		383.0	107.0	1.21	0.0080	57	I
122	protriptylene	259.0	613.0	2.4	346.0	263.4	12.0	5.00	0.0002	90	I
123	puromycin	11.5	35.6	3.1		471.5	160.9	0.27	na	na	
124	quinidine	36.4	990.0	27.2		495.1	45.6	2.79	0.0096	80	*h*
125	reserpine	68.1	253.0	3.7		608.7	117.8	3.72	0.1000	50	*h*
126	ripseridone	389.0	627.0	1.6	536.0	410.5	59.7	2.58	na	70	
127	ritonavir	15.8	852.0	53.9		721.0	145.8	4.94	40.0000	68	IV
128	saquinavir	1.5	395.0	261.6	220.0	670.9	4.7	4.72	0.3604	4	III
129	paclitaxel	1.3	135.0	108.0		853.9	185.8	4.73	na	6	
130	terfenadine	65.6	306.0	4.7	285.0	471.7	43.7	6.07	0.2400	70	*h*
131	trimethoprim	73.9	267.0	3.6	110.0	290.3	105.5	0.98	8.0000	97	*h*
132	verapamil	415.0	718.0	1.7	440.0	454.6	64.0	4.47	0.0039	28	I
133	vinblastine	10.0	232.0	23.2		811.0	154.1	3.19	na	5	
134	vincristine	2.4	14.9	6.3		825.0	171.2	3.16	na	na	
135	vinorelbine	2.5	176.0	69.8		793.0	133.9	4.07	na	27	
136	zolmitriptan	2.5	6.3	2.5	2.2	287.4	57.4	1.23	0.0200	91.5	III

[a] Drug was considered as P-gp substrate (P-gpS) when it showed ER > 1.5 or positive to calcien AM and ATPase assays. [b] MDRI-MDCKII monolayer bidirectional permeability as reported.[12,13] [c] Represents passive permeability (absorptive transport of P-gpS in the presence of 2 μM GF120918, a specific P-gp inhibitor). [d] Calculated by eq 1 using solubility data from standard references.[15,18,19] [d] Mean values of % HIA obtained from individual drug references or from standard compilations.[14−17] [f] The solubility criterion for the BCS was based on Do with a cutoff of Do $\leq$ 1 for high solubility and Do $\geq$ 2 for low solubility. The permeability criterion from monolayer transport was set as $P_{app,AB} \leq 20$ nm·s^{-1} for low permeability and $P_{app,AB} \geq 100$ nm·s^{-1} for high permeability. [g] Not available. [h] Borderline class.

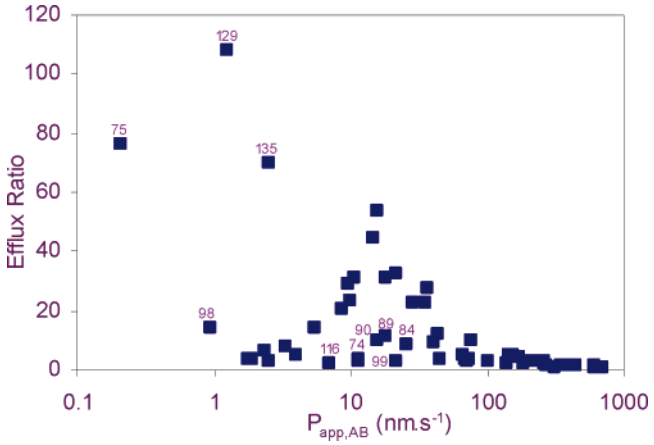

Figure 2. Relationship between MDRI-MDCKII monolayer permeability and the efflux ratio of P-gpS listed in Table 2. Note: Efflux ratio of saquinavir (~261) was not included in this plot for clarity. Numbers in the plot indicate the corresponding serial numbers of compounds given in Table 2.

Further, P-gpS have been classified into (i) category I, substrates with AQ ≥ 0.5; (ii) category II, substrates with AQ < 0.5 and SQ > 2; and (iii) category III, substrates with AQ < 0.5 and SQ < 2.

Figure 3a shows the relationship between passive permeability of P-gpS ($P_{PD,AB}$) and the functional activity (AQ) of P-gp. A parabolic relationship, however with a few outliers, was found, where P-gpS exhibiting 150 < $P_{PD,AB}$ < 425 nm·s^{-1} fall into category I and are highly influenced by P-gp efflux. This trend once again substantiates the hypothesis that moderately permeable P-gpS demonstrate significant absorption limitations attributable to P-gp-mediated efflux. For a drug that is a substrate for active transporters, the relative contribution of active transport will depend upon the concentration of the substrates at the enterocytes. Further, a linear relationship was found between AQ and $P_{app,AB}$, except for a set of dugs (Figure 3b). As AQ increases, transport is more attenuated with P-gp and thus $P_{app,AB}$ decreases. Exceptional drugs (acrivastine, cetirizine, cimetidine, famciclovir, labetolol, neostigmine, pirenzapine, trimethoprim (see circle of Figure 3)) to this trend are less permeable and also demonstrated less attenuation with P-gp. These compounds are less permeable due to their intrinsic passive transport, where P-gp-mediated efflux is of little significance. The linear relationship further indicates that AQ better predicts the P-gp-mediated drug efflux and may be used in absorption prediction models incorporating the functional role of P-gp.

Lipinski's Rule-of-5 and Passive Permeability of NS and P-gpS. Passive permeability via the transcellular and paracellular pathways is controlled primarily by the physicochemical properties of a drug (lipophilicity, MW, charge state, and hydrogen bonding). Lipinski's rule-of-5 states that poor absorption or permeability is more likely when the passively permeable molecules have (i) *Clog P* >5, (ii) MW >500, (iii) hydrogen bond donors >5, and (iv) hydrogen bond acceptors >10.[27] Analyses of physicochemical profiles

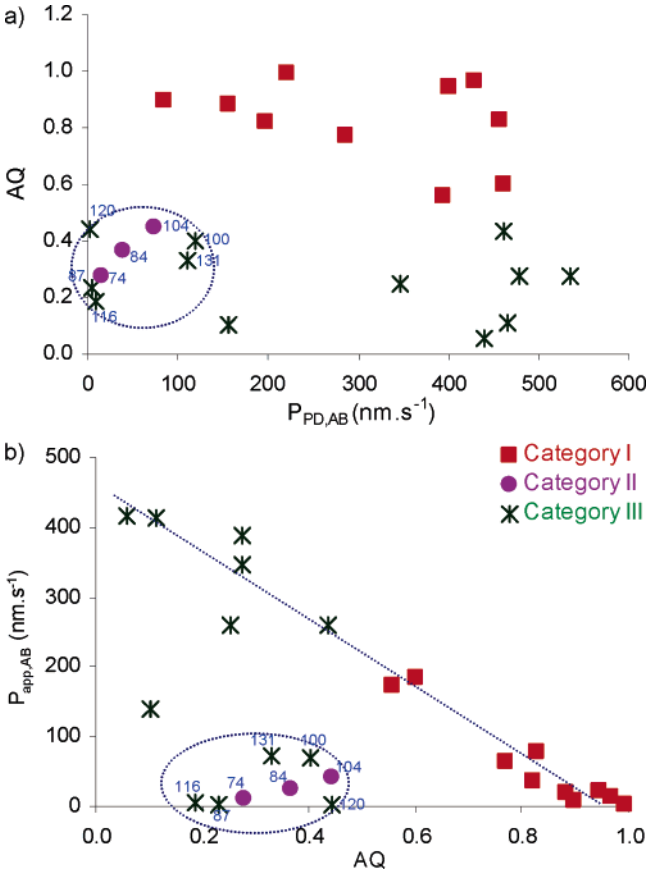

Figure 3. (a) Plot of P-gpS passive permeability, $P_{PD,AB}$ (in the presence of inhibitor), versus AQ. (b) Plot of AQ versus overall absorptive transport, $P_{app,AB}$, of P-gpS. Drugs in the dotted circle are exceptions from the linear relationship of AQ versus $P_{app,AB}$. Numbers in the plot indicate the corresponding serial numbers of compounds given in Table 2.

(*Clog P*, PSA, and MW (Tables 1 and 2)) of the two datasets are given in Figure 4. NS has a parabolic relationship between *Clog P* and $P_{app,AB}$ in MDRI-MDCKII cell monolayers, with drugs having high permeability when *Clog P* is more than 1. This is in agreement with Kasim et al.[28] *Clog P* limits, based on which WHO essential drugs and U.S. top 200 drugs have been classified into the BCS. It is expected that, at higher lipophilicity, solubility-limited absorption limits bioavailability; however, it should be noted that permeability falls as *Clog P* goes to more than 5 (Figure 4). Because of the lipidic nature of membrane bilayer, lipophilicity better correlates to the passive permeability of drugs. Drugs with low *Clog P* show limited diffusion into the phospholipids of the cell membrane, while drugs with

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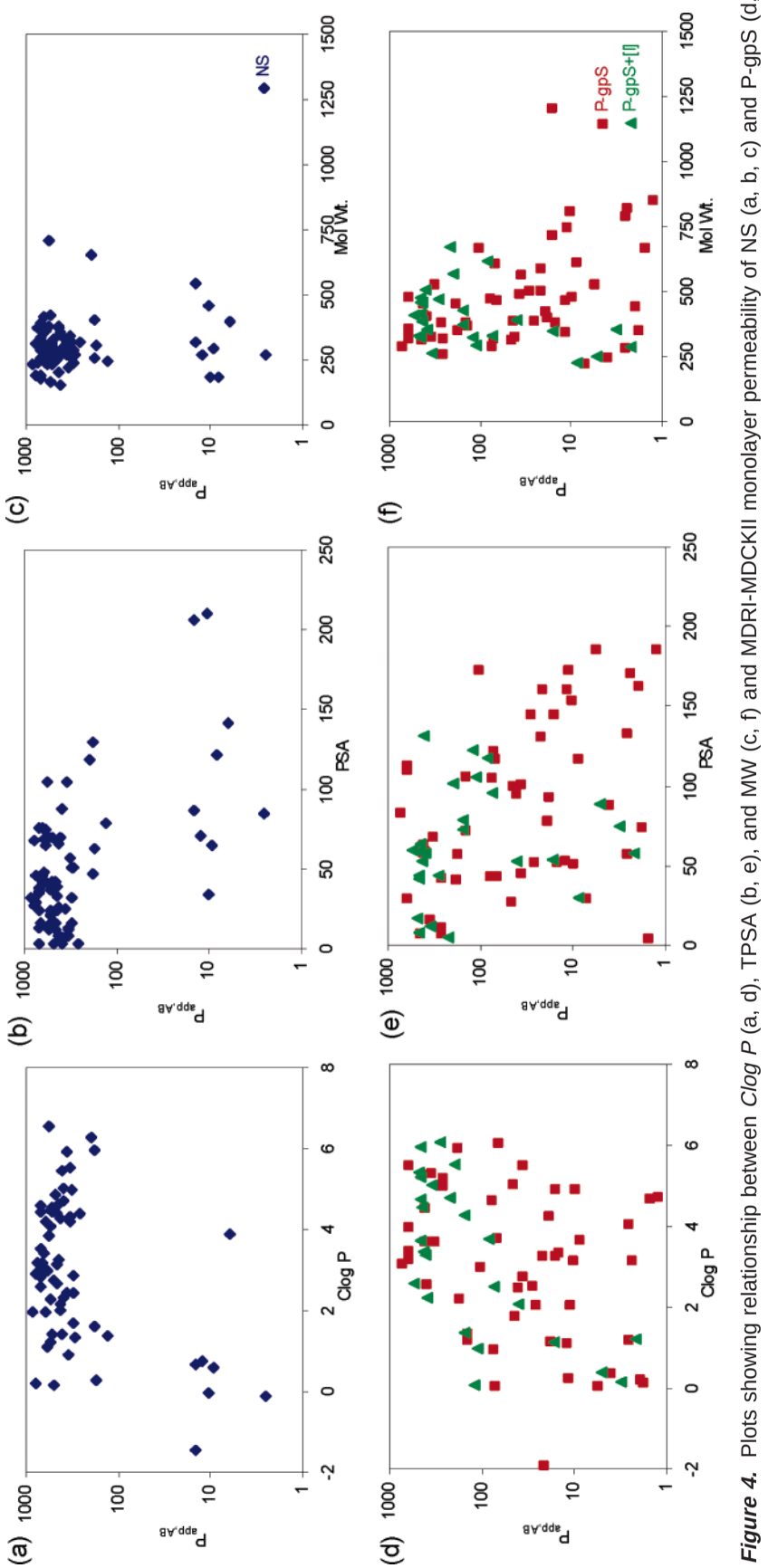

Figure 4. Plots showing relationship between *Clog P* (a, d), TPSA (b, e), and MW (c, f) and MDRI-MDCKII monolayer permeability of NS (a, b, c) and P-gpS (d, e, f).

high *Clog P* (>5) show preferential partitioning into phospholipid cell membranes preventing passage through the aqueous portion of the membrane. It is pertinent to note that most of the P-gpS have *Clog P* in the desired range for high passive permeability but showed less permeability because of high attenuation by P-gp. This hypothesis may be substantiated by P-gp inhibition studies, where most of the P-gpS falling in the *Clog P* range of 1−5 have improved permeability in the presence of 2 μM GF120918.

TPSA has been suggested as an easily assessable descriptor for hydrogen bonding and provides a better relationship to permeability.[29] Permeability versus TPSA plots a sigmoidal relationship with NS (Figure 4). However, it is observed that the descending part covers a relatively wide range of TPSA. Arbitrarily, for drugs with TPSA >75 Å^2, both NS and P-gpS showed less permeability. The mean TPSA values for NS and P-gpS are 49.74 and 92.35 ($p < 0.001$), respectively. From the dataset, a significant difference ($p < 0.001$) was also found between the mean MWs of NS (302.7) and P-gpS (488.4). P-gpS are toward the higher limits of MW with about 30% of molecules showing >500, while only 4% of NS have MW >500. Taking MW as a representative molecular size descriptor and TPSA as a hydrogen-bonding parameter, it can be concluded not only that permeability limitations of these P-gpS, as they violate Lipinski's rule-of-5, are due to transport attenuation by P-gp but also that, for a number of molecules, unfavorable physicochemical profiles limit the passive permeability. As discussed earlier, drugs with moderate passive permeability are more attenuated by P-gp (Figures 2 and 3). It may be concluded that unfavorable physicochemical properties of P-gpS limit passive permeability, leading to more susceptibility to efflux. It is also interesting to note that P-gpS with MW >500 and TPSA >100 Å^2 have an average ER of 49.5, indicating that molecules with such physicochemical properties are highly influenced by P-gp efflux. Another tentative conclusion that can be drawn is that drugs with high MW and TPSA are more likely to be P-gpS, apart from having intrinsic poor passive permeability. Admittedly, there is no direct evidence; however, from the analysis of the P-gpS dataset it may be inferred that P-gpS must have H-bond-donating and/or -accepting structural features (high TPSA) for effective interaction with P-gp.

Quantitative BCS of NS and P-gpS. Drugs listed in the datasets were classified to the BCS on the basis of the Do and MDRI-MDCKII permeability, which are taken as indicative of fundamental properties of drug absorption, solubility, and permeability (Tables 1 and 2). The criterion for solubility was kept as unity (Do = 1), where the maximum dose strength is soluble in 250 mL of water and the drug is in solution form throughout the GI tract. The criterion is more conservative for solubility classification, and it was extended to two (Do = 2) for borderline classification, considering the average volume of fluid (500 mL) under fasting conditions.[30] Irvine et al. using a set of 55 compounds showed an approximately sigmoidal relation between $P_{app,AB}$ and HIA and also demonstrated a linear correlation of MDCK and Caco-2 permeability values.[20] On the basis of the relationship among MDCK permeability, Caco-2 permeability, and HIA, a cutoff for highly permeable drugs, $P_{app,AB} = 100$ nm·s^{-1}, ensuring >90% bioavailability with a borderline cutoff of 20 nm·s^{-1} has been set. Drugs with permeability in the range of 20−100 nm·s^{-1} were considered as borderline drugs. Overall, the classification of drugs was based on the recent proposals and definitions of solubility criteria[28] and permeability criteria.[31]

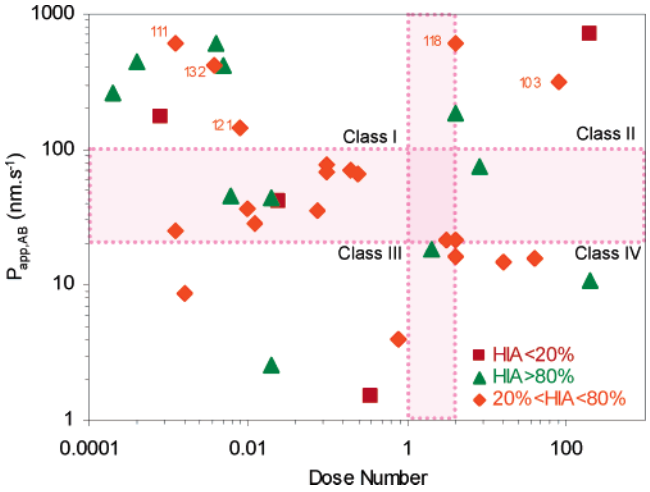

Figure 5. Classification of P-gpS with low (<20%), moderate (20−80%), and high (>80%) HIA into the BCS based on Do and monolayer permeability. The solubility criterion for the BCS was based on Do with a cutoff of Do ≤ 1 for high solubility and Do ≥ 2 for low solubility. The permeability criterion from monolayer transport was set as $P_{app,AB} \leq 20$ nm·s^{-1} for low permeability and $P_{app,AB} \geq 100$ nm·s^{-1} for high permeability. Numbers in the plot indicate the corresponding serial numbers of compounds given in Table 2.

Among the 63 P-gpS, 24 (38%), 14 (22%), and 25 (40%) molecules showed the characteristics of highly permeable, borderline, and low-permeability classes, respectively (Table 2). Of the data available, 70% of P-gpS are incompletely absorbed (<80% HIA), where P-gp-mediated efflux may be involved with many drugs (Table 2 and Figure 5). In general, P-gpS which are incompletely absorbed showed MDRI-MDCKII monolayer permeability <100 nm·s^{-1}. However, a few drugs (e.g., verapamil, ketoconazole, midazolam, nifedipine, and prazosin) with high absorptive permeability are incompletely absorbed, which can be attributed to reasons other than P-gp-mediated efflux, as these compounds showed

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(30) Yu, L. X.; Amidon, G. L.; Polli, J. E.; Zhao, H.; Mehta, M. U.; Conner, D. L.; Hussain, A. S. Biopharmaceutics classification system: the scientific basis for biowaiver extensions. *Pharm. Res.* **2002**, *19*, 921−925.

(31) Rinaki, E.; Valsami, G.; Macheras, P. Quantitative biopharmaceutics classification system: the central role of dose/solubility ratio. *Pharm. Res.* **2003**, *20*, 1917−1925.

low P-gp activity as evident from their efflux parameters. It is also interesting to observe that inhibition of P-gp by 2 μM GF120918 showed improvement of permeability for drugs falling into the borderline permeability class, moving most of them to class I or II. Thus, a pharmacokinetic advantage is more likely for many P-gpS on P-gp modulation, especially for compounds with moderate permeability.

The main objective of the BCS is to predict in vivo performance of drug product from in vitro measurements of solubility and permeability.[32] On the basis of the features of P-gpS from the present dataset, the functional role of P-gp in limiting oral absorption and the implications of P-gp inhibition on the biopharmaceutics and pharmacokinetics of P-gpS and the possible absorption related drug interactions may be discussed with the principles of the BCS, as follows.

Class I, Do $\leq$ 1, $P_{app,AB} > 100$ nm·s^{-1}. Drugs belonging to class I are highly soluble and highly permeable, where for passively permeating P-gpS, high P_{PD} overrules the efflux effect of P-gp. Inhibition or induction of P-gp does not show a significant effect on the pharmacokinetics of P-gpS belonging to this class. Absorption-related drug interactions are less likely. However, increasingly the contribution of the intestinal mucosa to first-pass metabolism is being recognized. For example, midazolam, a highly permeable P-gp substrate with $P_{app,AB}$ of 609 nm·s^{-1}, has only 36% oral bioavailability because of first-pass metabolism, of which 43% occurs in intestinal cells during absorption.[33] Examples: chlorpromazine and nicardipine.

Class II, Do > 2, $P_{app,AB} > 100$ nm·s^{-1}. P-gpS belonging to class II are fairly permeable and may be well absorbed in the duodenum and proximal jejunum, where P-gp expression is less.[34,35] However, due to the low solubility, the absorption site is shifted more towards the distal intestine, where P-gp effect may be pronounced. Overall, saturation of P-gp by providing high drug concentrations at the site of absorption or P-gp inhibition by using P-gp modulators may significantly improve the pharmacokinetics of these drugs. Examples: ketoconazole and mebendazole.

Class III, Do $\leq$ 1, $P_{app,AB} < 20$ nm·s^{-1}. These drugs either have less intrinsic permeability due to their unfavorable physicochemical properties or are strong substrates to efflux transporters, or both. Even though a class III drug is available in high concentrations at the site of absorption, low permeability leads to complete access to P-gp at subsaturation levels. In actual in vivo conditions, since most of the dose of less permeable drugs is absorbed from the lower intestine, the effect of P-gp is pronounced and thus the pharmacokinetics of these drugs are highly influenced by P-gp inhibition and/or GI transit. Examples of class III P-gpS include indinavir, emetine, and saquinavir.

Class IV, Do > 2, $P_{app,AB} < 20$ nm·s^{-1}. Low absorption for these drugs is anticipated because of the combined limitation of solubility and permeability. These drugs are more likely susceptible to P-gp efflux as the concentration of the drug in the enterocytes at any given time will be less to saturate the transporter. Inhibition of P-gp provides a scope for improving deliverability of molecules. Examples: paclitaxel, eletriptan, and clarithromycin.

Borderline Class, 1 < Do < 2, 20 < $P_{app,AB}$ < 100 nm·s^{-1}. Most of the P-gp substrates fall in the permeability borderline limits (Figure 5); thus inhibition of P-gp has a profound effect on the overall BA of these drugs. Drugs with both solubility and permeability in the borderline region will be highly attenuated by P-gp and are also influenced by GI transit.

Conclusions

In the present study, a large dataset of P-gpS was compared to the NS with respect to the permeability characteristics and Lipinski's rule-of-5. MDR1-MDCKII cell monolayer permeability showed a distinct difference in the permeability characteristics of P-gpS and NS, and a large number of P-gpS showed limited permeability as a result of P-gp efflux. The efflux parameter, AQ, correlated well to the P-gp activity in limiting intestinal permeability of drugs. A distinct difference in the MW and TPSA properties was found between P-gpS and NS, with P-gpS preferentially distributed towards higher MW and TPSA, which could be attributed to their less intrinsic passive transport. P-gpS demonstrating high passive permeability were found to be least influenced by P-gp, while transport of drugs with moderate passive permeability is highly attenuated by P-gp. Thus, integration of P-gp efflux parameters with the characteristics of the BCS class provides better absorption predictions.

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