MDCK (Madin–Darby Canine Kidney) Cells: A Tool for Membrane Permeability Screening

JENNIFER D. IRVINE,* LORI TAKAHASHI, KAREN LOCKHART, JONATHAN CHEONG, JOHN W. TOLAN, H. E. SELICK, AND J. RUSSELL GROVE

Contribution from *Affymax Research Institute (a Glaxo Wellcome Company), Santa Clara, California 95051.*

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Abstract □ The goal of this work was to investigate the use of MDCK (Madin–Darby canine kidney) cells as a possible tool for assessing the membrane permeability properties of early drug discovery compounds. Apparent permeability (P_{app}) values of 55 compounds with known human absorption values were determined using MDCK cell monolayers. For comparison, P_{app} values of the same compounds were also determined using Caco-2 cells, a well-characterized in vitro model of intestinal drug absorption. Monolayers were grown on 0.4-μm Transwell-COL membrane culture inserts. MDCK cells were seeded at high density and cultured for 3 days, and Caco-2 cells were cultured under standard conditions for 21 to 25 days. Compounds were tested using 100 μM donor solutions in transport medium (pH 7.4) containing 1% DMSO. The P_{app} values in MDCK cells correlated well with those in Caco-2 cells ($r^2 = 0.79$). Spearman's rank correlation coefficient for MDCK P_{app} and human absorption was 0.58 compared with 0.54 for Caco-2 P_{app} and human absorption. These results indicate that MDCK cells may be a useful tool for rapid membrane permeability screening.

Introduction

The rise of combinatorial chemistry and other drug discovery technologies has vastly increased the number of new compounds to be evaluated as potential drug candidates. Accordingly, new high-throughput strategies are required to evaluate compound properties beyond potency and selectivity. A major focus in the pharmaceutical industry is to develop new drugs with good oral bioavailability. One essential factor of oral bioavailability is the ability of a compound to be well absorbed in the small intestine. Because of its inherent simplicity compared to in vivo and in situ studies, there has been significant interest in the in vitro Caco-2 cell model of human intestinal drug absorption.

When cultured on semipermeable membranes, Caco-2 cells, derived from a human colon adenocarcinoma, differentiate into a highly functionalized epithelial barrier with remarkable morphological and biochemical similarity to small intestinal columnar epithelium.[1−3] Fully differentiated cell monolayers can be used to assess the membrane transport properties of novel compounds.[4,5] In addition, the apparent permeability (P_{app}) values obtained from Caco-2 transport studies have been shown to correlate to human intestinal absorption.[6,7] As a consequence, the Caco-2 cell monolayer model has proven extremely useful not only as a tool for mechanistic studies of drug absorption but also as an absorption screening assay for preclinical drug selection.[8−10]

Though well-characterized and time-proven, the Caco-2 assay remains a relatively low throughput method, due in part to the limitations of its 3-week growth period and regular maintenance feeding requirements. Proprietary culture conditions that greatly accelerate the Caco-2 monolayer differentiation rate, and hence reduce the required culture time, are commercially available, but at additional expense.[11] To reduce the tissue culture time, cost, and effort required for permeability testing, we chose to investigate whether a faster-growing cell line might be suitable for absorption screening.

MDCK (Madin−Darby canine kidney) cells are a common model for studying cell growth regulation, metabolism, and transport mechanisms in distal renal epithelia.[12−17] Like Caco-2 cells, MDCK cells have been shown to differentiate into columnar epithelium and to form tight junctions when cultured on semipermeable membranes.[18,19] The use of the MDCK cell line as a model cellular barrier for assessing intestinal epithelial drug transport was discussed by Cho et al.[20,21] Ranaldi et al. published P_{app} results from MDCK and Caco-2 testing of antimicrobial compounds, showing similar P_{app} values with both cell lines.[22,23] Others have pointed out that MDCK cells are a good candidate for modeling simple epithelia.[24] We chose to study a large number of compounds in both MDCK and Caco-2 assays to evaluate the suitability of MDCK cells as a possible tool for assessing membrane permeability.

Materials and Methods

Materials—Cell culture media and buffer components were purchased from Gibco BRL (Gaithersburg, MD). Fetal bovine serum (FBS) was heat-treated at 56 °C for 30 min prior to use. Caco-2 and MDCK cells were obtained from the ATCC (American Type Culture Collection, Rockville, MD). Transwell-COL tissue culture inserts (6.5 mm diameter, 0.4 μm pore size, collagen-coated PTFE (poly(ethylene terephthalate)) were purchased from Costar Corporation (Cambridge, MA). An EndOhm volt-ohm meter and electrode were purchased from World Precision Instruments (Sarasota, FL). Amoxicillin was from Fluka (Ronkonkoma, NY). Cephalexin monohydrate and loracarbef-D monohydrate were from USP (Rockville, MD). Practolol was from Tocris Cookson (St. Louis, MO). Zidovudine (AZT) was from Aldrich Chemical Company (Milwaukee, WI). Compound 0311C90, acrivastine, fluparoxan, lamotrigine, netivudine, ondansetron, sumatriptan succinate, and trimethoprim were received from Glaxo-Wellcome (Research Triangle Park, NC). Gabapentin was from Parke-Davis (Ann Arbor,

* Corresponding author. Telephone: (408) 522-5885. Fax: (408) 481-0393. E-mail: jennifer_irvine@affymax.com.

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MI). Olsalazine disodium was a gift from Pharmacia & Upjohn (Kalamazoo, MI). Sotalol hydrochloride was a gift from Irotec Labs (Little Island, Cork, Ireland). All other chemicals were from Sigma Chemical Company (St. Louis, MO).

Cell Culture—Caco-2 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 1% nonessential amino acids, and 2 mM fresh L-glutamine. Cells were cultured at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. Cells were passaged at 80−90% confluence (every 3−4 days) using Trypsin-EDTA solution (Gibco #25300−047). Culture inserts were preincubated with culture medium (1 h, 37 °C) and then seeded with 63 000 cells per cm^2 (0.33 cm^2 per insert). Caco-2 monolayers were fed with fresh medium 24 h after seeding and then 3 times per week. Caco-2 monolayers were cultured for 21−25 days before use. Caco-2 cells were used at passage number 31 to 42, after receipt at passage 18 from ATCC.

MDCK cells were maintained in Minimal Essential Medium (MEM) containing 10% FBS and 2 mM fresh L-glutamine. MDCK cells were cultured and passaged in the same manner as Caco-2 cells. Culture inserts were preincubated with culture medium (1 h, 37 °C) and then seeded with 664 000 cells per cm^2 (0.33 cm^2 per insert). MDCK monolayers were washed and fed with fresh medium 1 h post-seeding and again 24 h post-seeding. MDCK monolayers were cultured for 3 days before use. MDCK cells were used at passage number 59 to 80, after receipt at passage 51 from ATCC.

Transport Assays—*Monolayer Screening*—Cell monolayers were fed with the appropriate culture medium on the day of assay. Two hours after feeding, monolayers were washed with transport medium (Hanks' Balanced Salt Solution (HBSS, Gibco #14025−092) + 10 mM Hepes, pH 7.4). Monolayers were equilibrated in transport medium for 30 min at 37 °C, in 95% humidity. The electrical resistance of each monolayer was measured at 37 °C at three locations using an STX-2 "chopstick" electrode and volt-ohm meter. The resistance of bare filter inserts was subtracted from monolayer resistance values. Monolayer resistance values were multiplied by the membrane area (0.33 cm^2) and averaged to calculate transepithelial electrical resistance (TEER ($\Omega \cdot cm^2$)) values for each monolayer. TEER values indicate the degree of monolayer confluence and tight junction development.

MDCK cells consistently showed lower TEER values than Caco-2 cells. Caco-2 monolayers with TEER values less than approximately 230 $\Omega \cdot cm^2$ were not used. MDCK monolayers with TEER values <90 $\Omega \cdot cm^2$ were not used. These cutoff values were determined to be the lower limit of the usual TEER value observed for each cell line. Typically, 70−90% of both MDCK and Caco-2 monolayers were acceptable for testing using this pre-screen procedure.

Transport Assay—Transport assay donor solutions consisted of 100 μM test compound in transport medium containing 100 μM lucifer yellow and 1% DMSO (pH 7.4). All test compounds were evaluated for suitable solubility and stability under assay conditions and a detection limit of ≤ 1 μM prior to assay. DMSO (1%) was used to increase the solubility of more hydrophobic compounds and was previously found to have no effect on Caco-2 paracellular or transcellular permeability of five test compounds. Ethanol and methanol were found to be unacceptable cosolvents for Caco-2 transport assays (unpublished results). Lucifer yellow, a fluorescent marker for the paracellular pathway, was used as an internal control in every test to verify tight junction integrity during the assay.

Transport assays were conducted using 0.3 mL of apical (AP) donor solution and 1 mL of basolateral (BL) acceptor solution (transport medium, pH 7.4). All compounds were tested in six replicate monolayers. Monolayers were incubated with donor and acceptor solutions for 60 min at 37 °C, 95% humidity, with 30 rpm reciprocal shaking. BL compartments were sampled at 15, 30, and 60 min. AP compartments were sampled at 60 min. The quality control compound set (a pool of propranolol, salicylic acid, cephalexin, vinblastine, and lucifer yellow) was assayed as a pool under the same conditions as the test compounds, using a donor solution containing 100 μM of each compound in transport medium (pH 7.4). Under these assay conditions, PEPT 1 dipeptide transporter activity was assumed to be minimal, because this transporter requires a pH gradient.

Most compounds, including the quality control compound set, were quantified by HPLC (Hewlett-Packard 1050 system) using a C18 column (YMC J'Sphere H80), variable wavelength detection (HP1050−VWD), and an acetonitrile/0.02 M ammonium formate, pH 3.7, mobile phase. Gabapentin was quantified via derivitization with dansyl chloride followed by fluorescence detection (Jasco 920 HPLC fluorescence detector; Ex = 352 nm, Em = 510 nm) using a Nucleosil C18 column and an acetonitrile/0.05M Tris HCl, pH 7.5, mobile phase. Mannitol was quantified by ion-exchange chromatography (Carbo-Pac PA10) with pulsed amperometry detection (Dionex ED40) using a gold electrode and a mobile phase of 52 mM NaOH. Sumatriptan was quantified using DC amperometry (Dionex ED40) with a carbon electrode and a Zorbax Elipse XDB−C8 column with a mobile phase of methanol/0.075 M phosphate (30:70) at pH 7.0. Lucifer yellow was quantified using a fluorescence 96-well plate reader (CytoFluor II (Biosearch, a subsidiary of Millipore)), Ex = 485 nm, Em = 530 nm.

Calculations—P_{app} (apparent permeability) values were calculated according to the following equation:

$$P_{app} = \left(\frac{dQ}{dt}\right) \times \frac{1}{C_0} \times \frac{1}{A} \quad (1)$$

where dQ/dt is the permeability rate, C_0 is the initial concentration in the donor compartment, and A is the surface area of the filter. Permeability rates were calculated by plotting the percent of initial AP drug mass (peak area) found in the BL compartment versus time and determining the slope of the line. The P_{app} values for most compounds were calculated from 15 min data to ensure that <10% of initial compound was found in the acceptor compartment. In some cases, no compound was detected in 15-min samples, so later timepoints were used. Loracarbef was detected in 30-min samples from both cell lines. Gabapentin was detected in 60-min samples from both cell lines. Olsalazine was detected in 30-min samples from MDCK cells and in 60-min samples from Caco-2 cells. Amoxicillin was detected in 60-min samples from Caco-2 cells.

Lucifer yellow (LY) results were used as an internal control for each monolayer to verify tight junction integrity during the entire assay period. Variations in tight junctions can significantly affect permeability results for compounds using the paracellular transport route. Accordingly, LY P_{app} values were quantified from 60-min basolateral samples after background subtraction. The normal range for LY permeability in Caco-2 monolayers observed in our study was approximately 1 to 7 nm/sec (1 nm/s = 1×10^{-7} cm/s). Results from Caco-2 monolayers with LY P_{app} > 10 nm/s were not used in compound P_{app} calculations. LY permeability in MDCK monolayers was unknown at the start of this study, though it was expected to be somewhat higher than in Caco-2 cells because of the lower resistance values observed for MDCK monolayers. Initially, results from MDCK monolayers with LY P_{app} > 30 nm/s were not used. Later in the study, the MDCK LY cutoff was adjusted to 15 nm/s.

Spearman's rank correlation coefficient, a measure of association between two separate rankings of n items, was calculated as follows:

$$r_s = 1 - \frac{6\sum d^2}{n^3 - n} \quad (2)$$

where d equals the difference between the two ranks that each item received, and n equals the number of items.[25] The value r_s is always between −1 and +1. Greater r_s values indicate greater positive association. Lower r_s values indicate less association.

Calculated log water:octanol partition coefficient (CLogP) values were calculated using Pcmodelsx, a hydrophobicity and polarizability prediction from Daylight Chemical Information Systems, Inc. (Mission Viejo, CA).

Quality Control—As a quality control measure to monitor the consistency of the cells during this experiment, a standard set of six compounds was tested at the beginning and end of the study with both cell lines. The standard set was composed of passive transcellular and paracellular compounds (propranolol HCl and LY), one dipeptide transporter substrate (cephalexin), and one *p*-glycoprotein substrate (vinblastine sulfate). Salicylic acid and cimetidine were also included, though the transport mechanisms of these compounds are somewhat more complex. The standard set was tested as a pool containing 100 μM each of the six compounds. The P_{app} of each compound in the pool was previously determined to be equivalent to the P_{app} of each compound tested

individually. The LY and TEER values were also monitored to ensure monolayer consistency during the study.

Results

Compound Test Set Assembly—To perform this study, a set of test compounds with known human absorption values (% absorption) and a broad range of physicochemical properties was assembled from literature references and Glaxo Wellcome data (Table 1). [6,7,26–33] Great care was taken to verify human intestinal absorption values by identifying primary references wherever possible. Primary references for most of the compounds tested here have been cited individually elsewhere.[33] Bioavailability data were not used. It was a challenge to identify compounds with low to intermediate levels of absorption (10 to 70%) because most compounds with known human absorption data have been successful as oral formulations. Compounds were not otherwise pre-selected in any manner that should bias the results of this study.

Compounds transported by more complicated transport mechanisms than passive diffusion were purposely retained in this set. Both Caco-2 and MDCK cell lines are known to express *p*-glycoprotein (Pgp) and dipeptide transporters, important mechanisms in the intestinal and renal barriers that retain nutrients while exporting xenobiotics. We were particularly interested to see how results for these compounds would compare between the two cell lines. Six compounds tested (amoxicillin, cefatrizine, cephalexin, gabapentin, lisinopril, and loracarbef) were reported dipeptide transport substrates. Three of the compounds tested (dexamethasone, ranitidine, and sulfasalazine) were reported efflux substrates.

MDCK and Caco-2 Monolayers—The formation of confluent MDCK monolayers with functional tight junctions was confirmed by microscopy, TEER values, and LY permeability results. The use of optically clear semipermeable membranes for culture (Costar Transwell-COL) allowed for visual examinations by microscopy. The TEER values provided a simple quick estimate of tight junction formation, which was then assessed more thoroughly by examining LY permeability. Results from a growth study showed evidence of tight junction maturation as TEER values increased and LY permeability decreased with time from zero to 3 days (Table 2). The average MDCK TEER value during this study was 173 $\Omega \cdot cm^2$ (± 51). The average MDCK LY P_{app} value was 6.3 nm/s (± 6.1).

As for MDCK cells, Caco-2 monolayer confluence and tight junction integrity were assessed by microscopy, TEER values, and LY permeability values. The average Caco-2 TEER value during this study was 280 $\Omega \cdot cm^2$ (± 50). The average Caco-2 LY P_{app} value was 3.1 nm/s (± 2.8).

Monolayer Quality Control—Quality control results with the standard set of six compounds indicated that both Caco-2 and MDCK cell lines produced similar permeability values at the beginning and end of the study (Table 4). The differences between the beginning and ending values were similar to the normal day-to-day variability for permeability values observed with these compounds.

MDCK P_{app} versus Caco-2 P_{app}—MDCK and Caco-2 permeability results are shown in Table 1. Values are reported as the average of replicates ± the standard deviation (SD). Included in the table are the reported human intestinal absorption values for the same compounds. When MDCK and Caco-2 P_{app} values were plotted against each other, a clear relationship was observed (Figure 1). The calculated r^2 value was 0.79, denoting a high level of correlation. Data for the known dipeptide transport and *p*-glycoprotein efflux substrates fit the Caco-2/MDCK correlation well. Greater deviation from the linear trend was seen among low P_{app} compounds. Some departure from linearity was noted for very high permeability compounds, where P_{app} values tended to be slightly greater in MDCK cells than in Caco-2 cells. The significance of this observation has not been determined.

Table 1—Permeability Results with MDCK and Caco-2 Monolayers

compound	human % abs[a]	P_{app} (nm/s)[c] MDCK	Caco-2
0311C90	60[b]	11 ± 4.1	29 ± 6.6
acebutolol HCl	90	17 ± 4.0	38 ± 2.6
acetaminophen	80	350 ± 16	1000 ± 57
acetylsalicylic acid	100	74 ± 20	22 ± 2.0
acrivastine	88	22 ± 7.8	19 ± 9.9
acyclovir	16[b]	2.1 ± 1.6	not detected
alprenolol HCl	93	1600 ± 84	1700 ± 98
amoxicillin	94	2.4 ± 0.9	0.21 ± 0.1
antipyrine	100	1500 ± 100	1500 ± 120
atenolol	50	18 ± 9.2	33 ± 4.4
AZT (Zidovudine)	100	60 ± 12	280 ± 69
bretylium tosylate	18	14 ±4.4	11 ± 1.4
bupropion HCl	87	1300 ± 74	1500 ± 220
cefatrizine propylene glycol	76	25 ± 13	7.6 ± 1.3
cefuroxime sodium	5	1.6 ± 1.6	3.8 ± 3.2
cephalexin monohydrate	98	4.8 ± 2.4	2.7 ± 0.8
chlorothiazide	13	3.0 ± 0.8	3.2 ± 1.2
corticosterone	100	1400 ± 32	1200 ± 200
dexamethasone	100	200 ± 17	400 ± 38
fluparoxan	100[b]	2500 ± 180	2000 ± 190
furosemide	61	6.2 ± 4.3	1.4 ± 0.1
gabapentin	50	3.6 ± 1.9	0.10 ± 0.04
guanabenz	75	1900 ± 280	1000 ± 78
hydrochlorothiazide	67	10 ± 2.8	9.2 ± 4.0
hydrocortisone	91	310 ± 14	560 ± 63
ketoprofen	100	200 ± 20	930 ± 100
labetalol HCl	95	250 ± 23	760 ± 240
lamotrigine	70	880 ± 56	1100 ± 350
lisinopril dihydrate	25	1.8 ± 3.7	2.2 ± 4.1
loracarbef-D monohydrate	100	9.1 ± 0.9	2.4 ± 0.8
mannitol	15	not detected	5.7 ± 1.9
methylprednisolone	82	160 ± 90	250 ± 52
metoprolol tartrate	95	1500 ± 97	1400 ± 100
nadolol	34	14 ± 4.3	3.9 ± 3.4
netivudine (882C87)	28[b]	14 ± 5.7	6.8 ± 1.1
olsalazine disodium	2	0.48 ± 0.24	0.16 ± 0.06
ondansetron	100	1100 ± 20	1100 ± 140
oxyprenolol HCl	90	1300 ± 49	1600 ± 250
penicillin V	45	1.5 ± 0.13	1.7 ± 0.34
phenytoin	90	1200 ± 54	1600 ± 180
pindolol	90	590 ± 26	960 ± 79
practolol	100	13 ± 2.6	61 ± 17
progesterone	91	1600 ± 80	980 ± 60
propranolol HCl	90	1700 ± 65	1100 ± 130
propylthiouracil	75	410 ± 130	960 ± 87
ranitidine HCl	50	not detected	not detected
salicylic acid	100	100 ± 37	130 ± 11
sotalol HCl	95	47 ± 8.0	42 ± 13
sulfasalazine	65	4.8 ± 4.1	6.0 ± 11
sumatriptan succinate	75[b]	19 ± 3.1	not detected
terbutaline hemisulfate	60	10 ± 2.7	4.1 ± 1.6
testosterone	100	1400 ± 110	1000 ± 71
timolol maleate	90	550 ± 34	1000 ± 80
trimethoprim	97	520 ± 89	870 ± 130
warfarin sodium	98	440 ± 39	960 ± 210

[a] Human % oral absorption values were obtained from published values. [b] Human % oral absorption values were obtained from Glaxo-Wellcome. [c] P_{app} values are mean ± standard deviation.

P_{app} vs Human Intestinal Absorption—When MDCK and Caco-2 P_{app} values were plotted against percent human absorption, an approximately sigmoidal relationship was observed with both cell lines (Figure 2). Well-absorbed compounds showed generally high P_{app} values, and poorly

Table 2—MDCK Monolayer Development

growth time[a]	TEER[b]	LY P_{app}[c]
2	≤1	830 ± 130
24	30 ± 3	150 ± 60
72	100 ± 8	≤5

[a] Hours. [b] Average TEER (Ω·cm²) of 4 replicates ± standard deviation. [c] Average P_{app} (nm/sec) of 4 replicates ± standard deviation.

Table 3—Spearman's Rank Correlation Coefficients (r_s)

comparison	r_s
Caco-2 P_{app} versus MDCK P_{app}	0.93
human absorption versus MDCK P_{app}	0.58
human absorption versus Caco-2 P_{app}	0.54
MDCK P_{app} versus cLogP	0.50
human absorption versus cLogP	0.25

Table 4—Quality Control Results for MDCK Validation Study[a]

cell	compound	start of study[a]	end of study[a]
Caco-2	cimetidine	15 ± 3.0	11 ± 2
	cephalexin	4.7 ± 1.0	1.9 ± 0.8
	salicylic acid	200 ± 7	120 ± 18
	propranolol HCl	600 ± 35	700 ± 35
	vinblastine SO_4	130 ± 10	120 ± 16
MDCK	cimetidine	15 ± 4	7.4 ± 3.1
	cephalexin	13 ± 9.8	5.9 ± 2.4
	salicylic acid	51 ± 5	37 ± 3
	propranolol HCl	1000 ± 81	830 ± 29
	vinblastine SO_4	72 ± 14	57 ± 5

[a] Average P_{app} (nm/s) ± standard deviation.

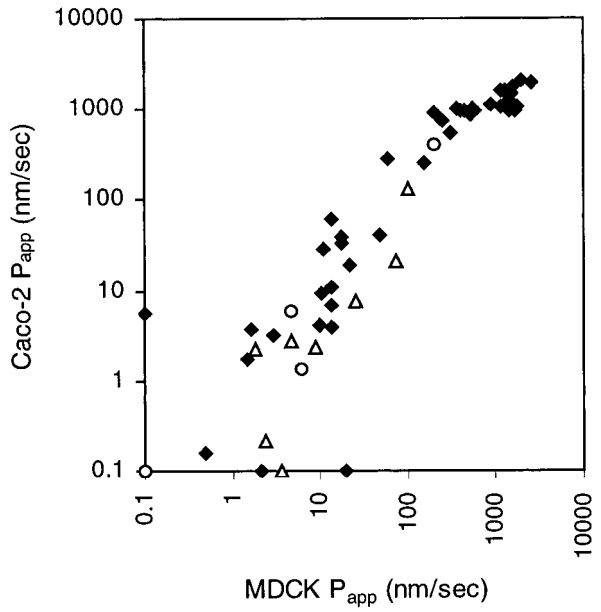

Figure 1—Correlation of MDCK and Caco-2 apparent permeability (P_{app}) values. P_{app} values plotted at 0.1 are actually ≤0.1, including zero for compounds not detected in the basolateral compartment. Key: (◆) = passive diffusion compounds; (△) active transport compounds; (○) efflux substrates.

absorbed compounds showed generally low P_{app} values. Some deviation from the sigmoidal trend was apparent with both cell lines, with somewhat greater deviation seen for Caco-2 data than for MDCK data. The compounds that fell out of the correlation were the same for both cell lines. Permeability values for amoxicillin, cephalexin, and loracarbef (three dipeptide transporter substrates) clearly underestimated their human absorption values of 94, 98, and 100%, respectively. Ranitidine (a *p*-glycoprotein efflux

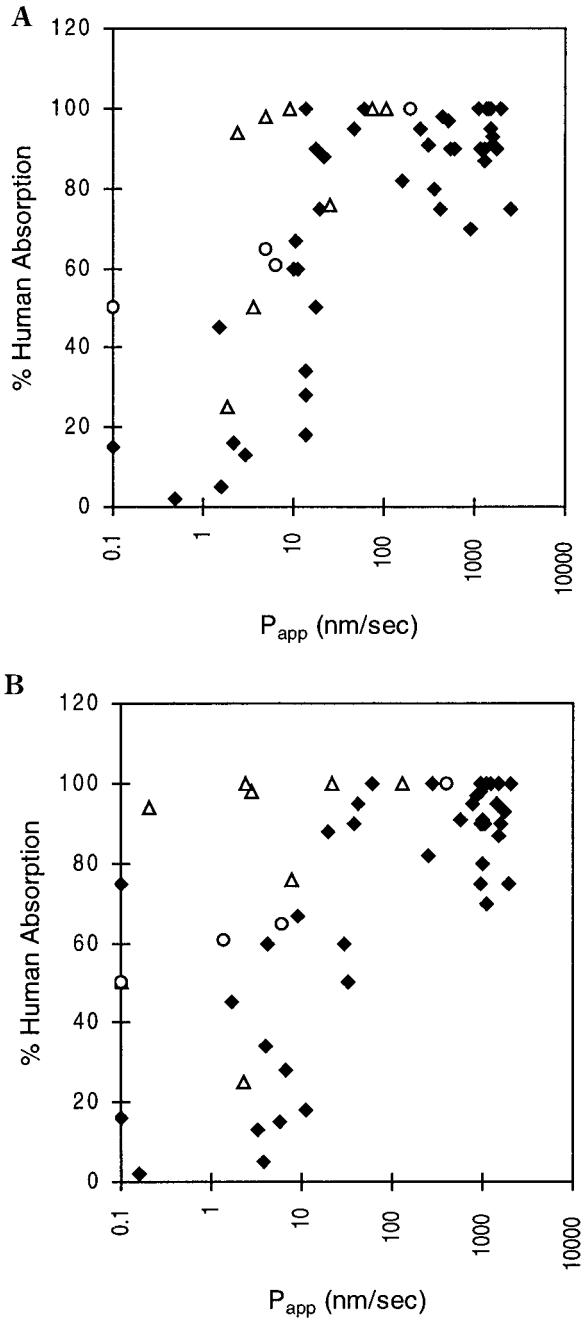

Figure 2—Correlation of apparent permeability (P_{app}) values and percent (%) human intestinal absorption. Figure 2A plots the relationship between MDCK P_{app} values and % absorption. Figure 2B plots the relationship between Caco-2 P_{app} values and % absorption. The P_{app} values plotted at 0.1 are actually ≤0.1, including zero for compounds not detected in the basolateral compartment. Key: (◆) passive diffusion compounds; (△) active transport compounds; (○) efflux substrates.

substrate) did not show detectable transport with either cell line, though it is reported to be 50% absorbed.

Rank Order—Because of the steep sigmoidal relationship between P_{app} values and human intestinal absorption and because of the variation in fit to the relationship, absolute absorption values are difficult to estimate from P_{app} data. In contrast, an estimate of rank order of absorption within a set of compounds, as measured by rank order of permeability, can be directly determined. Spearman's rank correlation coefficient was therefore calculated for MDCK and Caco-2 data to see how well either cell line modeled rank order of absorption (Table 3). The highest rank correlation was for MDCK P_{app} to Caco-2 P_{app} (0.93).

Rank correlations for MDCK P_{app} and Caco-2 P_{app} to human absorption were similar (0.58 and 0.54, respectively). In comparison, the rank correlation for cLogP to human absorption was 0.25. The rank correlation for human absorption to random number was calculated for perspective. For eight determinations, the average correlation was 0.0, however the value of one of the eight tests was 0.3.

Discussion

Although the MDCK cells are derived from dog kidney, whereas Caco-2 cells are derived from human colon, the two cell lines share many common epithelial cell characteristics.[15,17,34] That cells from such diverse origins could produce comparable transport results might seem surprising. Nonetheless, as a whole, we observed that permeability results from MDCK cells were similar to permeability results from Caco-2 cells. The r^2 value (0.79) for MDCK P_{app} versus Caco-2 P_{app} indicates the strength of that similarity, which is also visually evident in Figure 2. Because most of the compounds tested were passively absorbed, the good correlation between Caco-2 and MDCK P_{app} values suggests that the rules determining passive membrane permeability may be universal among cell types.

MDCK monolayers were seeded at a high density (664 000 cells/cm^2) to reduce tissue culture time. Similar high-density MDCK culture conditions have been reported by other labs.[14,34] MDCK TEER results in our studies (120−190 Ω·cm^2) were consistent with previously published data for cells seeded at a lower density and grown for 2−4 days.[20,24] Further studies in our lab have shown that the MDCK seeding density can be reduced by a factor of two or four without affecting assay performance (unpublished data). Caco-2 monolayers were seeded at a moderate density similar to those reported by other labs (63 000 cells/cm^2).[30,32] Caco-2 monolayers seeded at high density and assayed on day 3 showed high LY P_{app} values, which is evidence of poor tight junction integrity (unpublished results).

Permeability data from MDCK and Caco-2 monolayers correlated best for high P_{app} compounds. The weaker correlation observed for low P_{app} data may be partially due to greater variability in transport assay results for low permeability compounds and partially due to the magnification artifact of the log/log plot. We found permeability values of <10 tended to be poorly reproducible day to day. We therefore did not consider P_{app} results between 0.5 and 5.0 to be significantly different. Because of our interest in applying the permeation assay as a screening tool, that degree of error was tolerable.

Although most of the compounds tested here were absorbed by passive diffusion, to the best of our knowledge, some active transport compounds were included because oral absorption data was available. Caco-2 and MDCK P_{app} data for these active transport and efflux compounds appeared to correlate well. For the *p*-glycoprotein efflux substrates, the apical compound concentration may have been high enough to saturate the mechanism and negate any differences between the cells. For dipeptide transport substrates, both cell lines may have expressed roughly similar levels of transporters with roughly similar specificities. Caco-2 and MDCK permeability results for nonpassively absorbed compounds may not always correlate as well as observed here. This study was not designed to precisely examine and compare the permeability of active transport and efflux compounds in Caco-2 and MDCK cells. Although MDCK and Caco-2 cell lines have some active transport mechanisms in common, many transporters can affect drug permeability results.

The correlation between Caco-2 P_{app} values and human intestinal absorption that has been well-established by other laboratories was confirmed in these findings, and a similar correlation was observed for MDCK P_{app} values. In addition, both cell lines showed similar weaknesses with particular compounds. Thus, MDCK P_{app} values appear to function as well as Caco-2 cells when applied as a general absorption screen.

In conclusion, we have shown that drug permeability data from MDCK cells is similar to permeability data from Caco-2 cells, at least for passively absorbed compounds. We have found the fast-growing MDCK cell line can provide reliable results after only 3 days of culture, compared with the 21 days of culture required for Caco-2 testing. We suggest that it may be practical to use MDCK cells as a permeability screening tool to increase the throughput of membrane permeability screening in early drug discovery.

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