Research Article

Permeability Comparison between Hepatocyte and Low Efflux MDCKII Cell Monolayer

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Received 20 March 2014; accepted 7 May 2014; published online 23 May, 2014

Abstract. Determination of passive permeability is not only important for predicting oral absorption and brain penetration, but also for accurately predicting hepatic clearance. High throughput (HT) measurement of passive permeability across hepatocyte cell membrane is technically more challenging than using monolayer cell-based permeability assays. In this study, we evaluated if the HT Madin-Darby canine kidney II-low efflux (MDCKII-LE) cell monolayer permeability assay can be used as a surrogate to predict the passive permeability of hepatocytes. Apparent passive permeability of MDCKII-LE is well correlated to passive diffusion clearance of human and rat hepatocytes, suggesting that the HT MDCKII-LE assay can be used as a surrogate to estimate the passive permeability of hepatocytes. In addition, lipophilicity (Log D determined at pH 7.4) was also found to be well correlated with both MDCKII-LE and hepatocyte permeability for most compounds, hence it may serve as another permeability surrogate.

KEY WORDS: ADME; hepatocytes; Log D; MDCKII-LE; passive permeability.

INTRODUCTION

Passive permeability across hepatocytes is an important parameter impacting not only *in vivo* hepatic clearance, but also *in vitro* intrinsic clearance in hepatocyte systems for compounds with permeability-limited uptake into the hepatocytes. Accurate determination of a compound's passive permeability can assist in deconvoluting the various processes (active uptake, efflux transport, passive diffusion, and metabolism) that contribute to the hepatic clearance (1–3) and appropriate scaling factors can be applied as needed. This is particularly useful in understanding: (a) the rate-limiting step for hepatic clearance, (b) interplay between hepatic transporters and drug metabolizing enzymes, and (c) how the intrinsic clearance in hepatocytes compares to that in liver microsomes (this comparison has been shown to provide mechanistic information on cytochrome P450 (CYP) versus non-CYP metabolism and transporter effect (4)). Furthermore, such information is critical for liver targeting projects where compounds with low passive permeability and high active uptake by liver specific transporters (for example, organic anion

transporter polypeptide (OATP) 1B1 and 1B3) are identified in order to achieve high free drug concentration in the liver, while maintaining minimal systemic and peripheral tissue exposure to reduce toxicity (5–7). When predicting clinical outcomes using physiologically based pharmacokinetic models for transporter substrates and inhibitors, passive permeability across hepatocytes is an important input parameter to accurately model human pharmacokinetics, free liver drug concentration, and drug-drug interaction potentials (8).

There are several methods currently available to measure apparent passive permeability (P_{app}) or passive diffusion clearance (CL_{pass} , the product of permeability and cell surface area) across hepatocyte membranes. Most experiments measure the hepatocyte uptake rate in the presence of transporter inhibitors (e.g., rifamycin SV, inhibitor cocktail of cyclosporine A and rifampicin) or at low temperature (e.g., 4°C) in order to stop the active processes. These experiments use (a) plated hepatocytes, (b) hepatocytes suspension with oil spin technique, or (c) sandwich cultured hepatocyte systems (9,10). In general, the methods yield comparable passive permeability results, but there are some exceptions. For example, 4°C incubation can alter the membrane fluidity compared to incubation at 37°C, leading to temperature-dependent passive permeability (10). Transporter inhibitors, at times, can have substrate-dependent inhibition, making it difficult to interpret the data (10). The passive diffusion clearance in these studies is either estimated using the conventional two-step approach or the mechanistic two-compartment modeling approach (10–14). The mechanistic two-compartment model has the advantage of accounting for bidirectional passive diffusion while simultaneously assessing both uptake transport and metabolism (10). Additionally, if the assay is performed at multiple substrate concentrations, passive

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permeability can be estimated using a mechanistic model so that the 4°C incubation and transporter inhibitor are not needed (10,12). However, the method is quite laborious and requires a large amount of experimental data at varying time points. This approach uses 1-aminobenzotriazole (ABT) to inhibit CYP enzyme metabolism, which could also lead to inaccuracy in estimating permeability, due to incomplete inhibition of CYPs (15) and low selectivity towards other enzymes (16). In addition, when the mechanistic model is used without prior data transformation to delineate passive from active uptake into the cell, great uncertainty may be associated with the determination of passive permeability and intracellular unbound fraction (10). Overall, the hepatocyte-based methodologies are useful for determining passive and active processes, but they are relatively expensive, time and resource consuming, and low throughput. These assays are appropriate for profiling late-stage drug development candidates, but are less effective for early stage drug discovery projects with a large number of compounds from a variety of projects requiring rapid profiling.

The high throughput (HT) 96-well cell monolayer Madin-Darby canine kidney (MDCK) transwell assay has been widely applied in drug discovery to measure permeability of a large number of compounds. It would be highly desirable for predicting passive permeability across hepatocyte membranes in this HT format in order to increase throughput and reduce cost. Unlike other cell systems (e.g., human colonic adenocarcinoma (Caco-2) cells), Madin-Darby canine kidney II-low efflux (MDCKII-LE) was developed to have minimal endogenous transporter activity, making it a preferred system for measuring passive permeability (17). MDCKII-LE has been shown to adequately predict human oral absorption (17), but MDCK cells and hepatocytes are very different cell types (dog kidney cells versus human liver cells). Passive permeability values obtained from the MDCKII-LE cell monolayer have not yet been shown to predict passive permeability of hepatocytes. In this study, passive permeability across human hepatocyte membranes is compared with that obtained from the MDCKII-LE cell monolayer assay in order to evaluate if MDCKII-LE can be used as a surrogate for measuring hepatocyte passive permeability.

EXPERIMENTAL

Materials

Detailed information on materials for both MDCKII-LE and hepatocyte oil spin uptake experiments have been previously reported (9,17). Transwell insert plates (96-well) with polyethylene terephthalate membrane, 96-well angled-bottom collection plates, and velocity V11 peelable seals were purchased from BD Falcon (Bedford, MA). Deep 96-well plates were from Axygen Scientific Inc. (Union City, CA) and 96-tip blocks were obtained from Apricot Designs (Monrovia, CA). Test compounds were obtained from Pfizer Global Material Management (Groton, CT) or purchased from Sigma-Aldrich (St. Louis, MO).

Liquid Chromatography-Tandem Mass Spectrometry Conditions

The LC mobile phases were (or equivalent): (A) HPLC grade water containing 0.1% formic acid; and (B) acetonitrile

containing 0.1% formic acid. A solvent gradient from 5% (B) to 95% (B) over 2.0 min at the flow rate of 0.5 mL/min was used to elute the compounds from the column (Kinetex C18, 30×3 mm, 2.6 µm; Phenomenex, Torrance, CA). The cycle time was 3 min/injection. An aliquot of 15 µL was injected for analysis using a CTC PAL autosampler (LEAP Technologies, Carrboro, NC). The analysis was conducted with Shimadzu LC-10 AD HPLC pumps (Columbia, MD) connected to an AB SCIEX Triple Quad™ 5500 (Foster City, CA) mass spectrometer equipped with a TurboIonSpray source using MRM mode. Analyst™ 1.5.1 software (Applied Biosystems, Foster City, CA) was applied to data collection, processing, and analysis.

MDCKII-LE Monolayer Transport Assay

The experimental details of the MDCKII-LE assay have been previously described (17). A 96-well pipettor from Apricot Design PP550 (Monrovia, CA) was used for the assay. The assay was performed in 96-well format with a cassette of four test compounds per well (flux of four compounds is occurring simultaneously across a monolayer). Test compounds (2 µM) were added to the donor wells and buffer was added to the receiver wells. The plate was incubated (5% CO₂/95% O₂) at 37°C with 95% relative humidity. At 0 and 1.5 h time points, samples in both the donor and receiver compartments were collected for analysis.

The P_{app} values were calculated using Eq. 1.

$$P_{app} = \frac{1}{SA \cdot C_D(0)} \cdot \frac{dM_R}{dt} \quad (1)$$

where SA is the surface area of the cell monolayer (0.0625 cm²), $C_D(0)$ is the concentration in the donor at time 0, t is time in seconds, M_R is the mass of the compound appearing in the receiver as a function of time, and dM_R/dt is the rate of the compound accumulating in the receiver.

Human Hepatocyte Oil Spin Assay

Cryopreserved human hepatocytes (Celsis IVT, Baltimore, MD) were thawed at 37°C, then suspended in William's E media (WEM, custom formula #91-5233EC, GIBCO-BRL, Grand Island, NY). The suspension was aliquoted into two tubes and centrifuged at 50 g for 3 min at room temperature. The cell pellets were resuspended in regular Krebs-Henseleit buffer (KH buffer, Sigma-Aldrich, St. Louis). The resuspended cells were placed on ice for 10 min, then centrifuged and resuspended in KH buffer. The cell viabilities were determined by trypan blue exclusion and the suspensions were diluted to 2×10⁶ cells/mL. Cell suspensions of 200 µL with inhibitor (100 µM rifamycin SV), were aliquoted into test tubes and were prewarmed to 37°C in a slowly shaking water bath for 3 min. The incubations were initiated by the addition of 200 µL of 2 µM prewarmed substrate, with and without rifamycin SV, at 37°C, which resulted in the following final concentrations: 1 µM substrate, 100 µM rifamycin SV inhibitor and 1×10⁶ cells/ml cell density in

Table 1. Apparent Permeability (P_{app}) of MDCKII-LE and Passive Diffusion Clearance (CL_{pass}) of Suspended Human Hepatocytes and Literature CL_{pass} Values of Suspended, Plated, and Sandwich Culture Human and Rat Hepatocytes

Compounds	Charge	ACD ^a pK _a	MW (g·mol ⁻¹)	Measured Log D _{7.4} ^b	MDCKII-LE $P_{app} \pm SD$ (cm s ⁻¹ ·10 ⁻⁶) ^{**}	Hepatocyte CL_{pass} (μL min ⁻¹ mg ⁻¹ ·10 ⁻⁶)					
						Suspended human hepatocyte ± SD ^c		Suspended human Hepatocyte (13)		Plated human hepatocyte (12)	
						Conventional modeling	Mechanistic modeling	Mechanistic modeling	Mechanistic modeling	Conventional modeling	Mechanistic modeling
Bosentan	Anion	4.1	551	1.13	7.50 ± 1.62	1.69 ± 0.019	9.9	9.74	4.8	17.5	13.5
Cerivastatin	Anion	4.2, 5.6	459	1.76	12.2 ± 1.28	12.0 ± 0.30	—	—	25	23.7	12.0
Fexofenadine	Zwitterion	4.4, 9.4	502	0.427	0.397 ± 0.076	—	0.24	—	—	6.75	—
Fluvastatin	Anion	4.3	411	1.60	8.70 ± 0.282	13.2 ± 2.32	—	—	20	—	—
Glyburide	Anion	5.1	494	2.12	10.3 ± 1.53	—	100	—	—	—	—
PF-1	Anion	4.5, 7.2	503	1.26	4.94 ± 1.32	2.77 ± 0.71	—	—	—	—	—
PF-2	Anion	5.7	447	0.549	3.42 ± 1.23	0.511 ± 0.011	—	—	—	—	—
Pitavastatin	Anion	4.2, 4.7	421	1.20	5.65 ± 1.29	5.15 ± 1.39	13	13.2	—	18.8	10.9
Pravastatin	Anion	4.3	425	-0.840	0.440 ± 0.112	0.189 ± 0.11	0.7	0.208	0.1	1.67	1.29
Propranolol	Cation	9.5	259	1.09	19.0 ± 1.83	27.6 ± 9.22	—	—	—	—	—
Repaglinide	Anion	4.2, 5.8	453	2.08	16.1 ± 1.22	—	—	10	89	58.2	24.0
Rosuvastatin	Anion	4.3	481	-0.330	0.980 ± 0.480	0.797 ± 0.318	0.53	0.391	1.7	7.08	1.01
Telmisartan	Anion	3.9	515	2.22	11.9 ± 1.17	—	—	14.1	—	28.1	22.5
Valsartan	Anion	3.6, 4.2	435	-0.786	0.460 ± 0.186	0.330 ± 0.286	0.42	0.064	0.6	3.91	0.37

^a ACD (Version 12.5, Advanced Chemistry Development, Inc., Toronto, ON, Canada)

^b Pfizer internal data

^c Pfizer dataset

0.4 mL incubation volume. After an incubation period of 0.5, 1, and 1.5 min, 100 μ L incubation mixture was collected into a centrifuge tube (Denville Scientific Inc, NJ) containing an oil layer (density=1.015 g/mL, a mixture of silicone oil and mineral oil; Sigma-Aldrich, St. Louis, MO). The tubes were centrifuged at 14,000 rpm for 10 s (Beckman Microfuge E, Danvers, MA), then cut under the oil layer, and the cell pellets were lysed with 150 μ L of 70% methanol containing internal standard. The supernatant was analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The dimension of the hepatocytes was measured using Beckman Coulter Vi-CELL® XR Cell Viability Analyzer (Danvers, MA). The passive diffusion clearance (CL_{pass} , μ L/min/million cells) was estimated using the conventional model (Eq. 2).

$$CL_{pass} = \frac{v}{C(0)} \quad (2)$$

where v (pmol/min/million cells) is the initial rate of appearance of drug into the hepatocytes, and was calculated as the slope of the linear regression of the intracellular concentrations versus time plot. $C(0)$ is the initial substrate concentration (μ M).

Log D Determination

Log $D_{7.4}$ was determined using the previously described method (18). Test compound (10 mM DMSO stock, 2 μ L) was added to a deep 96-well plate containing 298 μ L of 50% 1-octanol and 50% phosphate buffer (pH 7.4) presaturated with one another. The assay was performed in duplicate with typical coefficient of variation (CV) ~20%. The plate was sealed and vigorously mixed on a plate shaker for 15 min at room temperature. The plate was then subjected to centrifugation at 2,500 rpm (1,006 $\times$ g) for 10 min to separate the phases. Aliquots from the 1-octanol phase and the buffer phase were removed from the wells, diluted accordingly and analyzed with LC-MS/MS.

Software

MATLAB (version 2012b; The MathWorks Inc., Natick, MA) with curve fitting toolbox (version 3.3) was used for all linear regression. ACD (version 12.5, Advanced Chemistry Development, Inc., Toronto, ON, Canada) was used to calculate pK_a and Log D.

RESULTS

A set of structurally diverse compounds, mostly OATP substrates, was selected for the study, because determination of passive permeability through hepatocytes is critical for modeling hepatic clearance of OATP substrates. Though compounds without transporter involvement may be better suited to evaluate the passive permeability component, it will not demonstrate if the approach will be applicable for OATP substrates. Determination of passive permeability and uptake rate is most important to predict PK and liver concentration for these classes of compounds. Both P_{app} from MDCKII-LE the monolayer transport assay and CL_{pass} values from the suspended human hepatocyte oil spin assay, along with their physiochemical properties, are summarized in Table I.

Literature CL_{pass} values from suspended, plated, or sandwich cultured human and rat hepatocytes are also included for the analysis (Table I).

A pure forward prediction on suspended human hepatocyte CL_{pass} (Pfizer dataset) from MDCKII-LE using the following equation (Eq. 3) was performed.

$$CL_{pass} = P_{app} \times 2 \times SA_{HHEP} \quad (3)$$

where the factor of 2 reflects the fact that compounds have to diffuse across two membrane layers (apical and basal membranes) before reaching the receiver compartment in the MDCKII-LE assay and only one layer of membrane for the hepatocyte assay. SA_{HHEP} is the surface area of one million human hepatocytes. Each human hepatocyte is assumed to be a sphere with a diameter of 13.52 μ m (measured). The predicted CL_{pass} values are similar to the observed values (Fig. 1) with squared Pearson's correlation coefficient of 0.815.

A linear regression between MDCKII-LE P_{app} and suspended human hepatocyte CL_{pass} (Pfizer dataset) on Log₁₀ scale was performed. The adjusted coefficient of determination (R^2) is 0.792 (Fig. 2a) and the regression result is shown in Eq. 4.

$$\text{Log}_{10}(CL_{pass}) = \text{Log}_{10}(P_{app}) \times 1.145 + 6.59 \quad (4)$$

where the unit for CL_{pass} is μ L/min/ 10^6 cells, and the unit for P_{app} is cm/s. Linear correlations were also observed between suspended human hepatocyte CL_{pass} (Pfizer dataset) and Log $D_{7.4}$ with adjusted R^2 of 0.818 (Fig. 2b, $R^2=0.694$ with propranolol), as well as MDCKII-LE P_{app} and Log $D_{7.4}$ with adjusted R^2 of 0.957 (Fig. 2c, $R^2=0.778$ with propranolol and fexofenadine). In both cases, propranolol and fexofenadine were outliers and excluded from the analysis. The regression results with Log $D_{7.4}$ are shown in Eq. 5.

$$\begin{aligned} \text{Log}_{10}(CL_{pass}) &= \text{Log}D_{7.4} \times 0.6032 - 0.1509 \\ \text{Log}_{10}(P_{app}) &= \text{Log}D_{7.4} \times 0.4773 - 5.843 \end{aligned} \quad (5)$$

Fig. 1. Correlation between observed and predicted suspended human hepatocytes passive diffusion clearance (HHEP CL_{pass} , Pfizer dataset). The red solid line represents diagonal line where predicted CL_{pass} =observed CL_{pass}

Fig. 2. Correlation among MDCKII-LE apparent permeability (P_{app}), suspended human hepatocytes passive diffusion clearance (HHEP CL_{pass}), and $\text{Log } D_{7.4}$ (Pfizer dataset). Circles represent observations, solid lines represent regression results, and squares represent compound excluded from the regression

Linear correlations were also observed between MDCKII-LE P_{app} measured in this study, and literature human hepatocyte CL_{pass} estimated using mechanistic two-compartment model (12–14) with adjusted R^2 of 0.889, 0.907, and 0.863 for the regressions with suspended, plated, and sandwich cultured human hepatocytes, respectively (Fig. 3a, Eq. 6).

$$\begin{aligned} \text{Log}_{10}(CL_{pass,suspended}) &= \text{Log}_{10}(P_{app}) \times 1.478 + 8.926 \\ \text{Log}_{10}(CL_{pass,plated}) &= \text{Log}_{10}(P_{app}) \times 1.434 + 8.229 \\ \text{Log}_{10}(CL_{pass,sandwich}) &= \text{Log}_{10}(P_{app}) \times 1.397 + 8.322 \end{aligned} \quad (6)$$

In addition, linear correlation was observed between mechanistically estimated human hepatocyte CL_{pass} from the literature and experimental $\text{Log } D_{7.4}$ with adjusted R^2 of

0.956 (R^2 of 0.721 with fexofenadine), 0.861, and 0.890 for the regression with suspended (13), plated (12), and sandwich cultured human hepatocytes (14), respectively (Fig. 3b, Eq. 7). Fexofenadine was an outlier and excluded from the analysis.

$$\begin{aligned} \text{Log}_{10}(CL_{pass,suspended}) &= \text{Log } D_{7.4} \times 0.7686 + 0.2309 \\ \text{Log}_{10}(CL_{pass,plated}) &= \text{Log } D_{7.4} \times 0.7216 - 0.1997 \\ \text{Log}_{10}(CL_{pass,sandwich}) &= \text{Log } D_{7.4} \times 0.7714 + 0.1113 \end{aligned} \quad (7)$$

Linear correlations were observed between MDCKII-LE P_{app} and literature rat hepatocyte CL_{pass} values with adjusted R^2 of 0.831 and 0.928 for the regression with suspended (11) and plated (10) rat hepatocytes, respectively (Fig. 4a, Eq. 8):

$$\begin{aligned} \text{Log}_{10}(CL_{pass,suspended}) &= \text{Log}_{10}(P_{app}) \times 0.6392 + 4.625 \\ \text{Log}_{10}(CL_{pass,plated}) &= \text{Log}_{10}(P_{app}) \times 1.066 + 6.563 \end{aligned} \quad (8)$$

A linear regression was performed between $\text{Log } D_{7.4}$ and literature rat hepatocyte CL_{pass} values. The adjusted R^2 values were 0.880 (R^2 0.878 with fexofenadine) and 0.906

Fig. 3. Correlation among MDCKII-LE apparent permeability (P_{app}), human hepatocytes passive diffusion clearance (HHEP CL_{pass}) estimated using the mechanistic model, and $\text{Log } D_{7.4}$. Red circles and solid lines represent observations and regressions with suspended hepatocytes, blue stars and dotted lines represent observations from plated hepatocytes, black triangles and dashed lines represent observations from sandwich cultured hepatocytes. The red square in **b** represents the data for fexofenadine which were excluded from regression between $\text{Log } D_{7.4}$ and CL_{pass} from suspended hepatocytes

Fig. 4. Correlation among MDCKII-LE apparent permeability (P_{app}), rat hepatocytes passive diffusion clearance (RHEP CL_{pass}), and $\text{Log } D_{7.4}$. Red circles and solid lines represent observations and regressions with suspended hepatocytes and blue stars and dotted lines represent observations from plated hepatocytes. The red square in **b** represents the data for fexofenadine which were excluded from regression between $\text{Log } D_{7.4}$ and CL_{pass} from suspended hepatocytes

for the regression with suspended (11) and plated (10) rat hepatocytes, respectively (Fig. 4b, Eq. 9).

$$\begin{aligned} \text{Log}_{10}(CL_{pass,suspended}) &= \text{Log } D_{7.4} \times 0.3755 + 0.7943 \\ \text{Log}_{10}(CL_{pass,plated}) &= \text{Log } D_{7.4} \times 0.5426 + 0.2924 \end{aligned} \quad (9)$$

For the suspended rat hepatocyte, a conventional model was employed (11) to obtain CL_{pass} , while for the plated rat hepatocyte, a mechanistic model was used (10).

For all the regressions above, no strong correlation was observed when the experimentally determined $\text{Log } D_{7.4}$ values were replaced by the predicted values from ACD (data not shown), probably due to the weak linear association between ACD predicted and experimentally determined $\text{Log } D_{7.4}$ values (Fig. 5).

DISCUSSION

Passive permeability is an important parameter of drug candidates. It has been widely applied in pharmaceutical research to predict oral absorption, brain penetration, skin permeation, and *in vitro* cellular uptake of drug molecules (19). Passive permeability across hepatocyte membranes impacts the intracellular free drug concentration of hepatocytes

Fig. 5. ACD predicted versus experimentally determined $\text{Log } D_{7.4}$. The red solid line represents diagonal straight line predicted $\text{Log } D_{7.4} = \text{observed } \text{Log } D_{7.4}$

since it modulates uptake of drug molecules into hepatocytes, metabolic clearance, and biliary elimination. The contribution of passive permeability to the overall hepatic intrinsic clearance ($CL_{int,app}$) can be described using the equation of sequential clearance (Eq. 10) (1–3), where $CL_{int,uptake}$, $CL_{int,pass}$, $CL_{int,met}$, $CL_{int,bile}$, and $CL_{int,efflux}$ are intrinsic clearance of active uptake, passive diffusion, metabolism, biliary clearance, and sinusoid efflux transport.

$$CL_{int,app} = (CL_{int,met} + CL_{int,bile}) \times \frac{CL_{int,uptake} + CL_{int,pass}}{CL_{int,met} + CL_{int,bile} + CL_{int,pass} + CL_{int,efflux}} \quad (10)$$

Accurate measurement of passive permeability is important in both *in vivo* PK prediction using mechanistic models, as well as understanding the contribution of the diffusion processes to *in vitro* intrinsic clearance by hepatocytes. A direct measurement of passive permeability across hepatocyte membranes is technically challenging because multiple mechanisms can occur in hepatocytes, including metabolism, influx by uptake transporters, efflux by efflux transporters, and passive diffusion. To measure the contribution of passive diffusion in such a complex system, the other processes would have to be inhibited or quenched without affecting passive permeability. The current approaches with inhibitors or low temperature (4°C) to stop the enzymatic and transporter processes have their limitations, and the mechanistic modeling approach (two-compartment model) is resource-intensive and low throughput. It is, therefore, necessary to develop a higher throughput and lower cost method to measure passive permeability of a larger number of compounds across hepatocytes. This is of particular interest for liver targeting drug discovery programs.

Since MDCKII-LE has already been widely used in drug discovery to measure passive permeability (17), it is natural to be considered as a surrogate for passive permeability of hepatocytes. However, there has been no evidence that the two systems have similar passive permeability, and the assay formats and cell origins are quite different as well. MDCKII-LE

cells are immortalized dog proximal tubule epithelial cells, whereas human hepatocytes are primary human epithelial cells. Based on these considerations, the correlation of passive permeability between the MDCKII-LE monolayer transport assay and various hepatocyte systems was evaluated, and relationship to lipophilicity ($\text{Log } D_{7.4}$) was also examined.

Potential limitations of comparing passive permeability between hepatocytes and MDCKII-LE directly (without converting to intrinsic membrane permeability) are the differences of cell membrane potential, paracellular transport and unstirred water layer (UWL) between the two systems. Membrane potential can impact the transmembrane transport of the ionized species of a compound, hence it affects P_{app} value (20). The effect of membrane potential was not corrected in this study because of the similarity of the voltages between the two cell types: -39 mV for (rat) hepatocytes (21) versus -48 mV for MDCKII-LE (22). Paracellular transport between the tight junctions of MDCKII-LE cells is believed to have minimal impact on P_{app} in this case (23), because all compounds, except for propranolol, are negatively charged at pH 7.4 and have molecular weight (MW) greater than 400. To evaluate the effect of UWL, MDCKII-LE P_{app} was estimated using partial differential equation-based mechanistic modeling (20). In the previous study (20) of UWL, it was suggested that compounds might have slower diffusion in the media, but faster depletion at the interface between media and membrane for highly permeable compounds. Hence, there was a shallow gradient across the membrane which decreased the mass transfer rate and resulted in UWL effect (20). However, in this study, using partial differential equation to model diffusion process in the media only yielded limited improvement over well-stirred media model (data not shown), possibly because most compounds under investigation have relatively low passive permeability. Therefore, a pronounced depletion effect at the media-membrane interface or UWL effect is less likely. As such, good correlation between MDCKII-LE P_{app} and human hepatocyte CL_{pass} was observed without correction of UWL (Figs. 1, 2a, and 3a). A slight overprediction shown in Fig. 1 might be due to inaccurately estimating effective cell surface area in the two assays (Eq. 1 and 3). The strong correlation between hepatocytes and MDCKII-LE (Figs. 1, 2a, and 3a,) indicated that MDCKII-LE can be used as a surrogate for passive permeability of hepatocytes.

The impact of human hepatocyte assay formats and modeling approaches to estimating passive permeability was evaluated using regression analysis with literature CL_{pass} of suspended (13), plated (12), and sandwich cultured human hepatocytes (14) using mechanistic modeling. In general, the regression results are similar among the different assay formats (Eq. 6), but are slightly different between the different modeling approaches (Eqs. 4 and 6).

Strong linear correlations were observed between $\text{Log } D_{7.4}$ and passive permeability of hepatocyte or MDCKII-LE among the Pfizer dataset, which is consistent with what was reported in the literature (10–12). This suggests that lipophilicity is a dominant factor for passive permeability of compounds tested in this study and $\text{Log } D_{7.4}$ can also be used as a surrogate for permeation across cell membranes. Propranolol was an outlier in both hepatocyte and MDCKII-LE comparisons with $\text{Log } D_{7.4}$ (Fig. 2b, c). $\text{Log } D_{7.4}$ underestimated the apparent permeability of both hepatocytes and MDCKII-LE (Fig. 2b, c), while MDCKII-LE predicted the passive permeability of hepatocytes

well for this compound (Figs. 1 and 2a). This could potentially be due to lysosomal trapping of the compound in hepatocytes, owing to the low pH of lysosomes (pH 4.7), high basicity (pK_a 9.5) and high lipophilicity of the compound (24). As a result, part of the uptake clearance of propranolol in hepatocytes, driven by lysosomal trapping, was not adequately described by $\text{Log } D_{7.4}$. For MDCKII-LE, lysosomal trapping should not impact P_{app} . However, paracellular permeation of propranolol can significantly contribute to P_{app} due to its low MW and positive charge at physiological pH. This might be one of the reasons that $\text{Log } D_{7.4}$ underpredicted the MDCKII-LE P_{app} . Fexofenadine, a zwitterion, was another outlier in the MDCKII-LE (Fig. 2c) and human hepatocyte (Fig. 3b) comparisons with $\text{Log } D_{7.4}$, although strong correlation was observed between human hepatocyte CL_{pass} and MDCKII-LE P_{app} (Figs. 1 and 2a). $\text{Log } D_{7.4}$ overpredicted the passive permeability of both hepatocytes and MDCKII-LE. This could potentially be due to the impact of cell membrane potential on permeation of charged molecules, which is not accounted for by $\text{Log } D_{7.4}$.

MDCKII-LE P_{app} values were also compared with literature suspended (11) and plated (10) rat hepatocyte CL_{pass} data (Table I and Fig. 4a). Although linear correlations were also observed between MDCKII-LE P_{app} and rat hepatocyte CL_{pass} , with different rat hepatocyte assay formats and modeling methods, the regression results are different (Fig. 4a, Eq. 8). The difference introduced by modeling methods has been published before (10), however, even with the same modeling method, plated, and suspended rat hepatocytes led to different CL_{pass} , particularly for low permeability compounds (10,11). Even though the suspended human and rat hepatocyte studies both used conventional method for data analysis, the actual experimental design and data fitting were quite different. This could be one of the reasons for the discrepancy between the two methods and this is an area requires further investigation to understand the factors impacting assay results. $\text{Log } D_{7.4}$ also described the rat hepatocyte passive permeability well (Fig. 4b, Eq. 9).

CONCLUSION

The results suggest that the MDCKII-LE P_{app} has a strong correlation with hepatocyte CL_{pass} . The MDCKII-LE assay can be used as a surrogate for passive permeability of human and rat hepatocytes. Since the MDCKII-LE assay is high throughput in a 96-well format and run in a four-in-one cassette, permeability values can be rapidly generated to develop structure-permeability or structure-transporter uptake rate relationships. Lipophilicity ($\text{Log } D_{7.4}$) has strong correlation with both hepatocytes and MDCKII-LE passive permeability for most compounds and can also be used as a permeability surrogate. More detail and comprehensive measurements of passive permeability with relevant systems and mechanistic modeling are still recommended for pharmacokinetic prediction of late-stage drug development candidates.

ACKNOWLEDGMENTS

The authors thank ADME Technology Group (ATG) for generating MDCKII-LE data and LC-MS/MS analysis, Keith Riccardi for providing the cell dimensions, Karen Atkinson for editing the manuscript, and Larry Tremaine for leadership and support.

REFERENCES

1. Yamazaki M, Suzuki H, Sugiyama Y. Recent advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. *Pharm Res*. 1996;13(4):497–513. doi:10.1023/A:1016077517241.
2. Shitara Y, Horie T, Sugiyama Y. Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci*. 2006;27(5):425–46. doi:10.1016/j.ejps.2005.12.003.
3. Watanabe T, Kusuha H, Sugiyama Y. Application of physiologically based pharmacokinetic modeling and clearance concept to drugs showing transporter-mediated distribution and clearance in humans. *J Pharmacokinet Pharmacodyn*. 2010;37(6):575–90. doi:10.1007/s10928-010-9176-y.
4. Di L, Keefer C, Scott Dennis O, Strelevitz Timothy J, Chang G, Bi Y-A, *et al*. Mechanistic insights from comparing intrinsic clearance values between human liver microsomes and hepatocytes to guide drug design. *Eur J Med Chem*. 2012;57:441–8. doi:10.1016/j.ejmech.2012.06.043.
5. Oballa RM, Belair L, Black WC, Bleasby K, Chan CC, Desroches C, *et al*. Development of a liver-targeted stearyl-CoA desaturase (SCD) inhibitor (MK-8245) to establish a therapeutic window for the treatment of diabetes and dyslipidemia. *J Med Chem*. 2011;54(14):5082–96. doi:10.1021/jm200319u.
6. Pfefferkorn JA, Guzman-Perez A, Litchfield J, Aiello R, Treadway JL, Pettersen J, *et al*. Discovery of (S)-6-(3-cyclopentyl-2-(4-(trifluoromethyl)-1H-imidazol-1-yl)propanamido)nicotinic acid as a hepatoselective glucokinase activator clinical candidate for treating type 2 diabetes mellitus. *J Med Chem*. 2012;55(3):1318–33. doi:10.1021/jm2014887.
7. Tu M, Mathiowetz AM, Pfefferkorn JA, Cameron KO, Dow RL, Litchfield J, *et al*. Medicinal chemistry design principles for liver targeting through OATP transporters. *Curr Top Med Chem (Sharjah, United Arab Emirates)*. 2013;13(7):857–66. doi:10.2174/1568026611313070008.
8. Poirier A, Cascais A-C, Funk C, Lavé T. Prediction of pharmacokinetic profile of valsartan in human based on *in vitro* uptake transport data. *J Pharmacokinet Pharmacodyn*. 2009;36(6):585–611. doi:10.1007/s10928-009-9139-3.
9. Kimoto E, Chupka J, Xiao Y, Bi Y-A, Duignan DB. Characterization of digoxin uptake in sandwich-cultured human hepatocytes. *Drug Metab Dispos*. 2011;39(1):47–53. doi:10.1124/dmd.110.034298.
10. Menochet K, Kenworthy KE, Houston JB, Galetin A. Simultaneous assessment of uptake and metabolism in rat hepatocytes: a comprehensive mechanistic model. *J Pharmacol Exp Ther*. 2012;341(1):2–15. doi:10.1124/jpet.111.187112.
11. Yabe Y, Galetin A, Houston JB. Kinetic characterization of rat hepatic uptake of 16 actively transported drugs. *Drug Metab Dispos*. 2011;39(10):1808–14. doi:10.1124/dmd.111.040477.
12. Menochet K, Kenworthy KE, Houston JB, Galetin A. Use of mechanistic modeling to assess interindividual variability and interspecies differences in active uptake in human and rat hepatocytes. *Drug Metab Dispos*. 2012;40(9):1744–56. doi:10.1124/dmd.112.046193.
13. Nordell P, Winiwarter S, Hilgendorf C. Resolving the distribution-metabolism interplay of eight OATP substrates in the standard clearance assay with suspended human cryopreserved hepatocytes. *Mol Pharmaceutics*. 2013;10(12):4443–51. doi:10.1021/mp400253f.
14. Jones HM, Barton HA, Lai Y, Bi Y-A, Kimoto E, Kempshall S, *et al*. Mechanistic pharmacokinetic modeling for the prediction of transporter-mediated disposition in humans from sandwich culture human hepatocyte data. *Drug Metab Dispos*. 2012;40(5):1007–17. doi:10.1124/dmd.111.042994.
15. Linder CD, Renaud NA, Hutzler JM. Is 1-aminobenzotriazole an appropriate *in vitro* tool as a nonspecific cytochrome P450 inactivator? *Drug Metab Dispos*. 2009;37(1):10–3. doi:10.1124/dmd.108.024075.
16. Sun Q, Harper TW, Dierks EA, Zhang L, Chang S, Rodrigues AD, *et al*. 1-Aminobenzotriazole, a known cytochrome P450 inhibitor, is a substrate and inhibitor of *N*-acetyltransferase. *Drug Metab Dispos*. 2011;39(9):1674–9. doi:10.1124/dmd.111.039834.
17. Di L, Whitney-Pickett C, Umland JP, Zhang H, Zhang X, Gebhard DF, *et al*. Development of a new permeability assay using low-efflux MDCKII cells. *J Pharm Sci*. 2011;100(11):4974–85. doi:10.1002/jps.22674.
18. Hay T, Jones R, Beaumont K, Kemp M. Modulation of the partition coefficient between octanol and buffer at pH 7.4 and pK_a to achieve the optimum balance of blood clearance and volume of distribution for a series of tetrahydropyran histamine type 3 receptor antagonists. *Drug Metab Dispos*. 2009;37(9):1864–70.
19. Kerns EH, Di L. Drug-like properties: concepts, structure design and methods: from ADME to toxicity optimization. London: Elsevier; 2008.
20. Ghosh A, Scott DO, Maurer TS. Towards a unified model of passive drug permeation I: origins of the unstirred water layer with applications to ionic permeation. *Eur J Pharm Sci*. 2014;52:109–24. doi:10.1016/j.ejps.2013.10.004.
21. Saito S, Murakami Y, Miyauchi S, Kamo N. Measurement of plasma membrane potential in isolated rat hepatocytes using the lipophilic cation, tetraphenylphosphonium: correction of probe intracellular binding and mitochondrial accumulation. *Biochim Biophys Acta Biomembr*. 1992;1111(2):221–30. doi:10.1016/0005-2736(92)90314-C.
22. Ishikawa T, Marunaka Y, Rotin D. Electrophysiological characterization of the rat epithelial Na^+ channel (rENaC) expressed in MDCK cells. Effects of Na^+ and Ca^{2+} . *J Gen Physiol*. 1998;111(6):825–46.
23. Sugano K. Introduction to computational oral absorption simulation. *Expert Opin Drug Metab Toxicol*. 2009;5(3):259–93. doi:10.1517/17425250902835506.
24. Kazmi F, Hensley T, Pope C, Funk RS, Loewen GJ, Buckley DB, *et al*. Lysosomal sequestration (trapping) of lipophilic amine (cationic amphiphilic) drugs in immortalized human hepatocytes (Fa2N-4 cells). *Drug Metab Dispos: Biol Fate Chem*. 2013;41(4):897–905. doi:10.1124/dmd.112.050054.