Cloe Screen MDR1-MDCK: A Predictive Model of Drug Permeability

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ABSTRACT

Madin Darby Canine Kidney cells transfected with the human *MDR1* gene (MDR1-MDCK), express the membrane transporter P-glycoprotein (P-gp). We have developed an MDR1-MDCK permeability screen for assessing the membrane permeability properties of early drug discovery compounds. This study measured the bi-directional transport of compounds with a range of permeabilities across MDR1-MDCK monolayers. Drug concentrations were analysed by LC-MS/MS, from which apparent permeability (P_{app}) values in apical-basolateral (A-B) and basolateral-apical (B-A) directions and asymmetry index (B-A P_{app} /A-B P_{app}) were calculated. Experiments were also carried out in the presence or absence of the P-gp inhibitor cyclosporin A. Results were compared with those from Caco-2 permeability studies as well as human intestinal absorption values and brain uptake classification obtained from literature. These results indicate that Cloe Screen MDR1-MDCK permeability assay is a useful predictive tool for assessing human intestinal absorption and uptake across the blood brain barrier in early drug discovery.

INTRODUCTION

Modern drug discovery technologies have resulted in a dramatic increase in the number of new compounds to be evaluated as potential drug candidates. This increase in the number of compounds early in the drug discovery process has resulted in the need for rapid and high quality methods to assess the Absorption, Distribution, Metabolism, Elimination, Toxicity (ADMET) and pharmacokinetic properties of these potential drug candidates. Determination of compound permeability and membrane transporter interactions of drug candidates is a key stage in lead selection and optimisation studies. Typically a combination of experimental models are used to measure drug permeability[1,2], often involving methods such as Parallel Artificial Membrane Permeability Assay (PAMPA), which utilises a hydrophobic filter coated with an artificial lipid membrane to assess passive permeation and cell-based approaches including Caco-2 and Madin Darby Canine Kidney (MDCK) cells for primary screening, followed by low throughput tissue-based approaches and whole animal models for secondary screening and mechanistic studies.

This study focused on the assessment of a permeability screen using MDR1-MDCK cells, and compared permeability results with those from Caco-2 cells, human intestinal absorption values and brain uptake classification. The wild type MDCK cells are an epithelial cell line of canine kidney origin and are suited to screen for passive permeation as they have low expression of transporter proteins and low metabolic activity. MDCK cell lines can be stably transfected with specific efflux transporters (e.g. MDR1, MRP2 and BCRP) in order to identify particular mechanisms of transport at the early stage of drug discovery. The most commonly used transfected cells are the MDR1-MDCK which express active P-glycoprotein (P-gp)[3], one of the most well characterised of the efflux proteins.

METHODS

1. Experimental protocol

MDR1-MDCK cells (NIH, Rockville, MD, USA) were seeded onto Multiscreen™ 96 well plates (Millipore, MA, USA) at 0.34 x 10^6 cells/cm^2, and cultured for 4 days for confluent cell monolayer formation. Dosing solution containing test compound at 10 μM in supplemented HBSS transport buffer at pH 7.4 was applied to the apical surface of the cell monolayer and compound permeation into the basolateral compartment measured (A-B). The assay was performed in the reverse basolateral to apical direction (B-A), to investigate P-gp-mediated efflux. The fluorescent integrity marker Lucifer yellow was also included in the dosing solution for both directions. Test compound permeability was assessed in duplicate over a 60 minute incubation at 37°C, 5% CO_2 with a relative humidity of 95%. Compounds were quantified by LC-MS/MS analysis using a 5 point calibration with appropriate dilution of the samples. A selection of compounds which demonstrated efflux were assayed in the presence and absence of the P-gp inhibitor, cyclosporin A (10 μM in the apical compartment).

2. Data Analysis

The apparent permeability coefficient (P_{app}) for each compound was calculated from the following equation:

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

Where dQ/dt is the rate of permeation of the drug across the cells, C_0 is the donor compartment concentration at time zero and A is the area of the monolayer.

An asymmetry index (AI) was calculated from the mean apical to basolateral (A-B) P_{app} and basolateral to apical (B-A) P_{app}:

$$AI = \frac{P_{app}\ (B\text{-}A)}{P_{app}\ (A\text{-}B)}$$

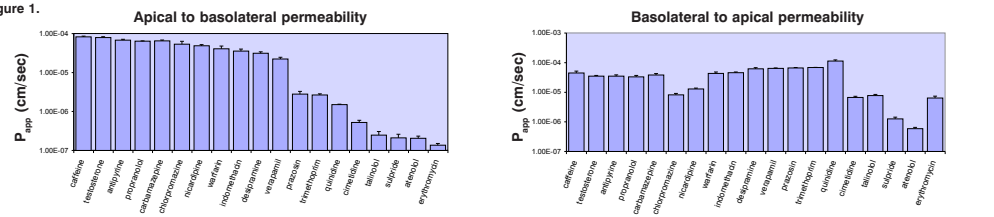
The entire process is automated so that 96 test compounds can be analysed in duplicate, in both directions on one day. We used a BasePlate™ liquid-handling robot (The Automation Partnership, Royston, UK), cassette LC-MS/MS analysis and a tailored laboratory-information management system.

RESULTS

Cloe Screen MDR1-MDCK Assay Validation

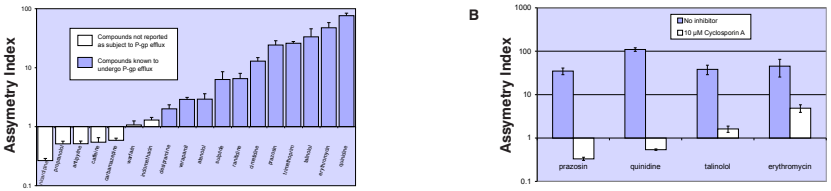
To assess intra- and inter-assay reproducibility, 19 compounds with a range of permeabilities were screened in the bi-directional assay in quadruplicate over three separate experiments. Figure 1 shows the mean of these assay runs and the error bars represent standard deviation. These data are highly reproducible (coefficient of variation < 20%) for a range of permeability values and over eight cell passages.

Figure 1.



Identification of P-gp Substrates Using Cloe Screen MDR1-MDCK Figure 2.A shows asymmetry indices calculated for compounds known to undergo P-gp efflux and those not reported as subject to P-gp efflux, for the mean of three assay runs and the error bars representing standard deviation. Figure 2.B demonstrates inhibition of P-gp-mediated efflux when four known substrates are co-incubated with 10 μM cyclosporin A (mean and standard deviations of quadruplicate incubations).

Figure 2.



Comparison with Caco-2 and Human Fraction Absorbed Data Experimental values of P_{app} A-B obtained with our Cloe Screen MDR1-MDCK and Cloe Screen Caco-2 permeability assays (Cyprotex Discovery, 2006), along with literature values for human intestinal fraction absorbed (Fa)[7] are shown in Figure 3. These demonstrate a good correlation (Caco-2 $r^2 = 0.90$, MDR1-MDCK $r^2 = 0.94$) over the range of human Fa values, indicating the predictive capabilities of these assays.

Classification of Brain Uptake The Cloe Screen MDR1-MDCK assay also clearly distinguishes between compounds which cross the blood brain barrier and those which do not. Table 1 shows P_{app} A-B of 14 compounds against CNS positive and CNS negative classification.

Figure 3.

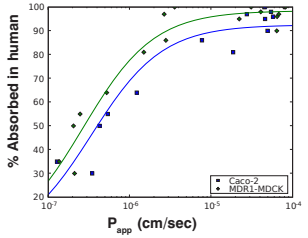


Table 1.

Drug	P_{app} A-B (x 10^{-6} cm/s)	Brain Uptake Classification
Atenolol	0.204	CNS Negative[2]
Methotrexate	0.234	CNS Negative[2]
Ranitidine	0.369	CNS Negative[2]
Vinblastine	0.521	CNS Negative[2]
Cimetidine	0.522	CNS Negative[4]
Sulfasalazine	0.535	CNS Negative[2]
Quinidine	1.49	CNS Negative[2]
Desipramine	31.1	CNS Positive[2]
Indomethacin	35.6	CNS Positive[2]
Warfarin	40.7	CNS Positive[2]
Chlorpromazine	53.4	CNS Positive[2]
Propranolol	63.9	CNS Positive[2]
Carbamazepine	64.5	CNS Positive[2]
Antipyrine	67.7	CNS Positive[2]

CONCLUSIONS

The Cloe Screen MDR1-MDCK permeability assay:

- Provides highly reproducible results and rapid data delivery
- Focuses on P-gp transport of drugs, which has been extensively studied and shown to be highly influential to the ADME characteristics of drug molecules[8,9] and so avoids the complexities of multiple transporters, as in Caco-2 cells
- Demonstrates excellent comparability to human intestinal absorption and blood brain barrier classification values
- Results from this assay can be used in conjunction with those from other assays, such as Cloe Screen Caco-2 and Cloe Screen PAMPA for a greater understanding of mechanisms of drug efflux and can highlight early potential issues with drug permeability

REFERENCES

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