Permeability Assays

Background

The Caco-2 cell line, derived from a human colorectal carcinoma, has become an established *in vitro* model for the prediction of drug absorption across the human intestine. When cultured on semi-permeable membranes, Caco-2 cells differentiate into a highly functionalized epithelial barrier with remarkable morphological and biochemical similarity to the small intestinal columnar epithelium. The membrane transport properties of novel compounds can thereby be assessed using these differentiated cell monolayers. The apparent permeability coefficients (P_{app}) obtained from Caco-2 cell transport studies have been shown to correlate to human intestinal absorption.[1,2]

Mandin Darby Canine Kidney (MDCK) cells are a common model for studying drug transport mechanisms in distal renal epithelia. Like Caco-2 cells, MDCK cells differentiate into columnar epithelium and form tight junctions when cultured on semi-permeable membranes. Primarily for passively absorbed compounds, drug permeability data from MDCK cell assays have been shown to be similar to permeability data from Caco-2 cell assays.[1] As a consequence, MDCK cells are gaining acceptance within the pharmaceutical industry as an alternate model to Caco-2 cells for rapidly screening compounds for absorption potential, as part of the pre-clinical drug selection process.

In addition, preliminary data from some laboratories show a good correlation between MDCK cell and Bovine Brain Endothelial Cell (BBEC) culture permeabilities for several compounds. BBEC cultures are used as an *in vitro* model to predict compound permeability across the blood brain barrier (BBB).

Key Features of the Assay

- model of drug absorption from the intestine to blood: mimicking the intestinal environment
- model of drug uptake from the blood into the brain: mimicking the blood brain barrier

Assay Applications

- mechanistic studies of drug absorption
- screening assay for pre-clinical drug selection

Diagram of assay insert

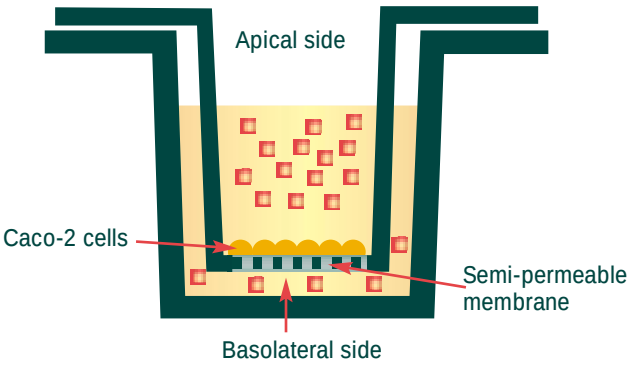

FIG. 1 Cell monolayers are cultured on a semi-permeable membrane to form tight cell-cell junctions. The inner well containing the cells is called the apical compartment while the outer well is called the basolateral compartment.

Assay Principle

Permeability across the Caco-2 or MDCK cell layer is determined by growing the cells on a membrane placed between two (donor and acceptor) chambers. Drug candidates are typically added to the apical side of the cell layer and their

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2820 Argentia Road, Unit 8,
Mississauga, Ontario L5N 8G4 CANADA
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www.noabbiodiscoveries.com

appearance in the basolateral side is measured over an incubation time. Permeability in this direction represents intestinal absorption. Permeability may also be determined from the basolateral to the apical side of the Caco-2/MDCK cells. A higher apical to basolateral P_{app}, compared to the basolateral to apical P_{app}, is indicative of carrier-mediated transport. P-gp mediated transport is suggested when a higher basolateral to apical P_{app} is observed.

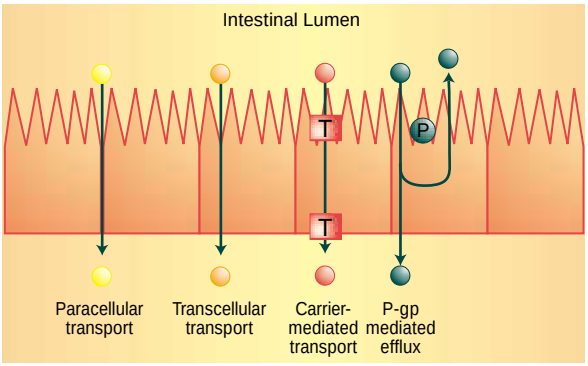

FIG. 2 The intestinal absorption of a compound may occur via passive diffusion (paracellularly or transcellularly), or via carrier-mediated active transport (eg. D-glucose, dipeptides). The intestinal absorption of a compound may be hindered by P-glycoprotein (P-gp), an ATP-dependent multidrug efflux pump. These pathways are also present for the permeation of compounds across the Caco-2/MDCK cell monolayer.

Assay Protocol

Caco-2/MDCK Cells

- seed cells in a semi-permeable membrane
- culture for predetermined time
- add drug to apical and/or basolateral compartments
- incubate for pre-determined time
- sample from each compartment at pre-determined times
- quantify drug concentrations by LC/MS/MS analysis

Typical Results

Compounds	P_{app} (nm/sec)		$P_{app\ (B\ to\ A)}/P_{app\ (A\ to\ B)}$
	Apical to Basal	Basal to Apical	
Caco-2 Cells			
Lucifer Yellow	11.3 ± 2.6	10.3 ± 2.5	0.91
Rhodamine 123	7.5 ± 1.5	21.4 ± 2.3	2.9
Warfarin	283 ± 19.4	269 ± 16.6	0.95
MDCK Cells			
Lucifer Yellow	30.8 ± 3.8	28.3 ± 4.0	0.92
Rhodamine 123	23.6 ± 3.8	48.8 ± 6.3	2.1
Warfarin	357 ± 11.2	326 ± 24.8	0.91

All values are mean ± SEM, N=7

This table shows the apparent permeability coefficient values derived in our laboratory for Caco-2 and MDCK cells. Passive diffusion of a substance occurs by the paracellular (lucifer yellow) or transcellular (warfarin) routes. Efflux of compounds by P-glycoprotein (P-gp), was demonstrated by rhodamine 123.

References

1. J.D Irvine et al. *J.Pharm.Sci*. 88:28 (1999)
2. P. Artursson. *J.Pharm.Sci*. 79:476 (1990)

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