

IN VITRO DRUG ABSORPTION

Intestinal epithelium permeability is a critical characteristic that determines the rate and extent of human absorption and ultimately affects the bioavailability of a drug candidate. Cerep's permeability assay allows a rapid assessment of membrane permeability and helps to rank-order compounds in terms of their absorption potential.

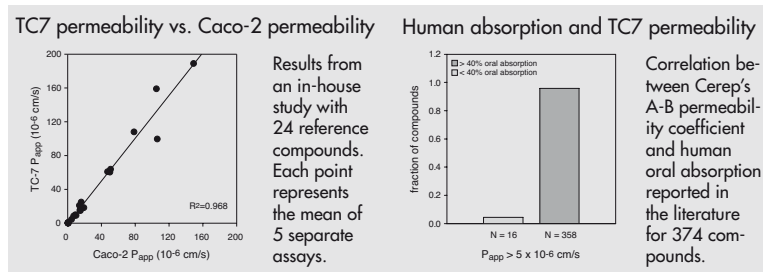
In addition, compounds that are possible substrates of P-glycoprotein (P-gp), an efflux pump located in the intestine and blood-brain barrier among other tissues, can also be identified using Cerep's permeability models.

ARE YOUR COMPOUNDS PERMEABILITY-LIMITED?

The absorption of orally administered drugs requires their movement across the intestinal epithelial barrier. Poor intestinal permeability leads to limited absorption. Generally, if a compound achieves a 90% or greater oral absorption, it is considered highly permeable (for example, propranolol or metoprolol). Drugs that display 50% or less oral absorption are considered poorly permeable (for example, ranitidine and atenolol). Compounds with extremely poor permeability are likely to have limited *in vivo* absorption. Permeability assessment in the early drug discovery stage will help to identify compounds that are likely to pose challenges during preclinical and clinical development.

IN VITRO INTESTINAL EPITHELIAL MODEL - THE CACO-2 CELL MONOLAYER

The Caco-2 cell line is a human colon adenocarcinoma cell line that differentiates in culture and resembles the epithelial lining of the human small intestine. It has been widely used as an *in vitro* intestinal epithelial model for drug transport and permeability screening of discovery compounds. A subclone of the Caco-2 cell line, TC7, is used as the *in vitro* intestinal model at Cerep for permeability assays. TC7 permeability correlates with the parental Caco-2 permeability and also correlates with human absorption (Figures 1 and 2). Furthermore, the apically-located efflux pump, P-glycoprotein (P-gp), is expressed in both the Caco-2 and the subclone TC7 cells.



CERE P'S PERMEABILITY ASSAYS

The apparent permeability coefficient (P_{app}) is determined in the apical-to-basolateral (A-B) and/or the B-A directions across cell monolayers cultured on 96-well polycarbonate membrane filters. Compounds are typically tested at 10 μ M with a final DMSO concentration of 1%. The assay plate is incubated at 37°C with gentle shaking. Samples are taken at time zero from the donor side and at the end of the incubation from both the donor and the receiver sides. Samples are analyzed by HPLC-MS/MS. The P_{app} value (expressed as 10⁶ cm/s) is then calculated based on the appearance rate of compound in the receiver side. In addition, recovery of the test compound (amount of compound detected in both the receiver and the donor sides at the end of the assay relative to the amount detected in donor side at time zero, expressed as percent) is also provided. The recovery value can be used to help interpret the P_{app} value. If the recovery is dramatically different from 100%, the P_{app} value should be interpreted with caution.

Four reference compounds (labetalol, propranolol, ranitidine, and colchicine) are tested concurrently in each permeability assay to ensure the validity of the assay. The P_{app} of a test compound can also be interpreted in comparison to that of the reference compounds, for which the human absorption data is known from the literature.

A-B PERMEABILITY

A-B PERMEABILITY (TC7, pH 6.5/7.4)

Test compounds are prepared at 10 μ M in HBSS-MES buffer, pH 6.5 and added to the apical side. HBSS-HEPES buffer, pH 7.4 is added to the basolateral side. Incubation is for 60 minutes. The slightly acidic pH (pH 6.5) in the apical side represents the average pH in the lumen of the small intestine, whereas the neutral pH (pH 7.4) in the basolateral side mimics the pH of the blood. Propranolol, is used as a highly permeable marker (≥ 90 % absorbed in human), labetalol is used as a medium permeable marker (≥ 90 % absorbed in human), ranitidine is used as a poorly permeable marker (about 50 % absorbed in human), and colchicine is used as a P-gp substrate (not used orally). Typical results of the reference compounds are shown in the table on the next page. Results from this assay allow rank-ordering of compounds and identification of compounds with potentially low absorption and bioavailability. Compounds with P_{app} values similar to or greater than that of propranolol are considered highly permeable and are likely to be "not permeability-limited". Compounds with P_{app} values similar to or less than that of ranitidine are considered poorly permeable and are likely to be "permeability-limited". Compounds with P_{app} greater than that of ranitidine and lower than that of propranolol are considered to have medium permeability. A suggested permeability classification with the permeability values from this assay is given as follows:

- $P_{app} < 2 \times 10^{-6}$ cm/s low permeability
- 2×10^{-6} cm/s $< P_{app} < 20 \times 10^{-6}$ cm/s medium permeability
- $P_{app} > 20 \times 10^{-6}$ cm/s high permeability

Cerep's permeability classification system and permeability results are in accordance with the FDA guidance "Waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System."

Typical permeability values (10^{-6} cm/s) for TC7 permeability assays ►

Compound (10 μ M)	A-B (pH 6.5/7.4)	A-B (pH 7.4/7.4)	B-A (pH 6.5/7.4)	B-A (pH 7.4/7.4)
labetalol	6.9	9.1	46.7	42.1
propranolol	48.3	63.3	30.4	18.1
ranitidine	0.5	0.9	5.2	5.3
colchicine	0.1	0.1	8.9	8.6

► A-B PERMEABILITY (TC7, pH 7.4/7.4)

Test compounds are prepared at 10 μ M in HBSS-HEPES buffer, pH 7.4 and added to the apical side. The same buffer without compound is added to the basolateral side. Incubation is for 60 minutes. In this assay, there is no pH gradient across the cell monolayer. Under this pH condition, any proton-driven transport activity is minimized. Therefore, this experimental condition allows more accurate examination of non-proton-driven transport mechanisms, such as P-gp mediated efflux (see more discussion under B-A Permeability section below).

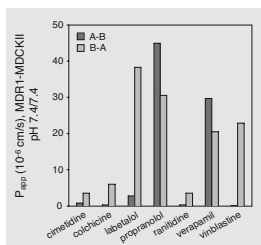
■ B-A PERMEABILITY

Several transport proteins that are expressed in human intestinal epithelium are also expressed in the Caco-2 cell model. While some transporters facilitate the absorption of compounds, efflux systems, such as P-gp, mediate the secretion of compounds from inside the cell back out to the apical medium (representative of the intestinal lumen), thereby limiting the overall absorption. In the B-A permeability assay, compounds are tested for secretion. In particular, test compounds are prepared at 10 μ M in HBSS-HEPES buffer, pH 7.4 and added to the basolateral side. The same buffer without compound is added to the apical side. The incubation is allowed for 40 minutes. This assay is typically performed together with the A-B permeability (TC7, pH 7.4/7.4) assay. The B-A P_{app} is compared to the A-B P_{app} for the same test compound. The ratio of B-A P_{app} to A-B P_{app} is designated as the efflux (E) ratio. Compounds with an E value greater than 5 are likely to be transported by one or more of the efflux systems and, as a result, their net absorption is most likely controlled by intestinal secretion. The permeability and E ratios obtained from Cerep's permeability assays for several known P-gp substrates are presented in the table.

Polarized permeability (10^{-6} cm/s) of known P-gp substrates in the TC7 cell model ►

Compound (10 μ M)	pH 7.4 in both A and B			pH 6.5 in A and pH 7.4 in B		
	A-B P_{app}	B-A P_{app}	E ratio	A-B P_{app}	B-A P_{app}	E ratio
colchicine	0.1	8.6	86	0.1	8.9	89
domperidone	4.4	57.3	13	3.2	70.1	22
vinblastine	0.4	40.2	100	0.2	10.0	50

■ ALTERNATIVE PERMEABILITY MODELS



The Madin-Darby canine kidney cell line (MDCKII) has been used as an alternative permeability model to the Caco-2 model. MDCKII is an epithelial cell line which originated from canine kidney.

Additionally, there is the MDR1-MDCKII cell line, which is the MDCKII cell line transfected with human MDR1 gene that encodes P-glycoprotein.

Currently, Cerep has validated permeability assays using both the MDCKII and the MDR1-MDCKII cell lines.

◀ MDR1-MDCKII permeability

■ CUSTOMIZATION

All assays can be customized as per the customer's specifications. Some examples of customization include test concentration, number of replicates, buffer pH, buffer type, and addition of P-gp inhibitor, among others.

■ QUALITY CONTROL OF CELL MONOLAYERS

Fluorescein is used as the cell monolayer integrity marker. Fluorescein permeability assessment (in the A-B direction at pH 7.4 on both sides) is performed after the permeability assay for the test compound. The cell monolayer that has a fluorescein permeability of less than 0.5, 1.5 and 2.5 ($\times 10^{-6}$ cm/s) for TC7, MDR1-MDCKII and MDCKII respectively, is considered intact.

■ ASSAY SELECTION GUIDE

Oral absorption

Drug absorption after oral administration is determined by several factors including the physical-chemical properties of the drug and its permeability across the intestinal epithelia. The following assays are typically used to evaluate the oral absorption of compounds.

- . A-B permeability (TC7, pH 6.5/7.4)
- . Aqueous solubility (PBS, pH 7.4)
- . Aqueous solubility (simulated gastric fluid)
- . Aqueous solubility (simulated intestinal fluid)
- . Partition coefficient (n-octanol/PBS, pH 7.4)
- . Chemical stability (simulated gastric fluid)
- . Chemical stability (simulated intestinal fluid)
- . *In vivo* PK in rats (IV and PO)
- . *In vivo* PK in mice (IV and PO)

Brain penetration (blood-brain barrier)

Brain penetration refers to the transport of a drug across the blood brain barrier (BBB) from blood to brain. The following are commonly used assays relevant for assessing the brain penetration potential of a compound.

- . A-B permeability (TC7, pH 7.4/7.4)
- . A-B permeability (MDR1-MDCKII, pH 7.4/7.4)
- . Partition coefficient (n-octanol/PBS, pH 7.4)
- . Partition coefficient (cyclohexane/PBS, pH 7.4)
- . *In vivo* BBB in rats
- . *In vivo* BBB in mice

P-glycoprotein mediated drug efflux

The following assays can be used to assess if a compound is a possible P-gp substrate.

- . A-B permeability (TC7, pH 7.4/7.4) \pm P-gp inhibitor ¹
- . B-A permeability (TC7, pH 7.4/7.4) \pm P-gp inhibitor ¹
- . A-B permeability (MDR1-MDCKII, pH 7.4/7.4) \pm P-gp inhibitor ¹
- . B-A permeability (MDR1-MDCKII, pH 7.4/7.4) \pm P-gp inhibitor ¹

¹ e.g. verapamil



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