Caco-2 Permeability

Caco-2 cells (a human colorectal adenocarcinoma cell line) have been used for many years as a model of intestinal drug permeability[1]. Cells are cultured for 21 days on a Transwell™ membrane to form a polarised monolayer with tight junctions between cells and the expression of efflux transporters including P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)[2].

In our standard, bi-directional, assay protocol compounds in Caco-2 buffer are added to the apical or basolateral side of the Caco-2 monolayer. Immediately following this addition, an aliquot of the dosing solution is sampled (t=0). Aliquots are also sampled from both apical and basolateral sides after a 2 hour incubation, and are diluted with organic solvent containing internal standard. Samples are then analysed by LC-MS and quantified against a matrix-matched standard curve.

The assay is also available in an adjusted format for P-gp and BCRP inhibitor (IC_{50}) or substrate assessment.

Standard Assay Conditions

- Caco-2 in 96 Well Format (21-24 day culture)
- Buffer: pH 7.4
  - Alternate pHs available upon request
  - Buffer can be modified to better accommodate poorly soluble compounds or those with high levels of non-specific binding
- 10 µM compound concentration
- 2 hour incubation, 37°C, 5% CO_2
- N=3 replicates
- Analysis by LC-MS with calibration curves
- Marker compounds included (low permeability, high permeability, P-gp and BCRP substrates).

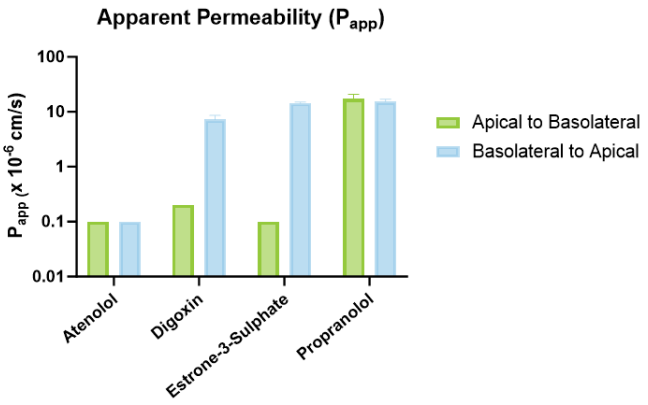

Figure 1: Bi-directional Apparent Permeability. N=3 experiments each with 3 replicates. Error bars denote standard deviation. Apical to Basolateral results for Atenolol and Estrone-3-Sulphate are <0.1 x 10^{-6} cm/s

Deliverables

- Excel summary of apparent permeability (P_{app}), efflux ratio and percentage recovery.

Compound Requirements

- 100 µL of a 10 mM DMSO stock or 1 mg sold (accurately weighed to 2 decimal places)

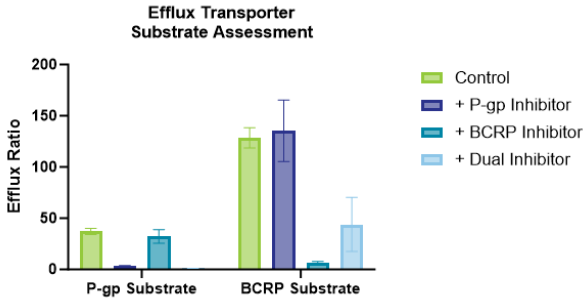

Figure 2: Data from our P-gp and BCRP substrate assessment assay. A decrease in efflux is observed in the presence of the relevant inhibitor. N=3 experiments each with 3 replicates. Error bars denote standard deviation.

References

1. Artursson &Karlsson (1991) Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells, Biochem Biophys Res Commun., 175(3), pp.880-885.
2. Jarc *et al* (2019) Demonstrating suitability of the Caco-2 cell model for BCS based biowaiver according to the recent FDA and ICH harmonised guidelines, Journal of Pharmacy and Pharmacology, 7(8), pp.1231-1242

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All our assays can be customised to answer specific project questions

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Contact us for more information on standard or customised assay formats