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CONCEPT LIFE SCIENCES

Concept Life Sciences:
Caco-2 permeability

Introduction

Caco-2 (an immortal human colon carcinoma cell line) is the most well known cell model for assessing in vitro permeability within drug discovery. The Caco-2 assay models the epithelial cell layer permeability barrier in the small intestine. Caco-2 cells develop microvilli on their apical surface that resemble the morphology of GI epithelial cells that line the intestinal villi. They also express cell membrane transporters on the apical surface, such as P-glycoprotein, BCRP and MRP2.

Deliverables: apparent permeability Papp, identify if efflux is present, and identify whether P-gp or BCRP are involved in transport.

Customer provides

Compound identifier and molecular formula.
Test: 30µLof 10mM in DMSO or 0.5mg solid.

Cells

Caco-2 cultured 21 days to form a monolayer on 24-well PET inserts. Integrity of monolayer con rmed with lucifer yellow < 1 x10-6 cm.s-1 and TEER > 350Ωcm2.

Test compound

Incubation concentration 10µM, n=2 (flexible).

Format

24-well plate, gentle orbital shaking incubator, 2hr at 37°C. Volumes used are 0.25mL apical, and 0.75mL basolateral. Use of shaker minimises the "unstirred water layer effect".

Protocol

Unidirectional transport assay: Test compound is added to apical well (gentle agitation) and transport determined by measuring concentration in basolateral well at 2hr (A→B).

Bidirectional transport assay: At the same time as A→B incubation, a parallel incubation is also performed where test compound is added to the basolateral well and then measured in the apical well (B→A) at 2hr. The efflux ratio is then calculated by comparing the rates of transport in both directions (B→A / A→B) to determine if test compound is an efflux transporter substrate.

P-gp or BCRP involvement: If efflux has been predetermined, then an additional study may be performed using Pgp or BCRP specific inhibitors that are co-incubated with test compound to establish if these transporters are involved.

The final solvent concentration is 0.1%DMSO.

For all assays after the 2hr incubation, 200µL aliquots are removed from all apical and basolateral wells and transferred to a new deep well plate to halt the transport.

- Aliquots are removed from the deep well plate and added to internal standard in a separate plate ready for LC-MSMS analysis.
- A second set of aliquots from the deep well plate representing the basolateral wells are removed and transferred to a separate plate ready for UV/vis absorbance to measure lucifer yellow.

Controls

Cimetidine and propranolol are used in each assay run. Lucifer yellow is used in donor wells and measured at the end of the experiment in the receiver wells to examine membrane integrity.

Quantitation

A standard curve is prepared for each test compound. Analysis is by LC-MS/MS. UV/vis absorbance at 492nm is used to measure lucifer yellow in basolateral against a standard curve.

Data analysis and results

$$\text{Response ratio} = \frac{\text{Test peak area}}{\text{Internal standard peak area}}$$

The response ratio is compared with the standard curve to determine the concentrations of the receiver and donor wells. The % recovery is determined to indicate the level of non-specific binding of the test compound, by comparing the concentrations with the initial concentration of the donor well.

The apparent permeability Papp is calculated according to

$$\text{Papp} = \frac{V}{A \times Co} \times \frac{dC}{dt}$$

Where V = receiver volume (cm3)
A = transwell membrane surface area (cm2)
Co = initial donor well concentration
dC/dt = rate of conce

$$\text{Efflux ratio} = \frac{\text{Papp (B to A)}}{\text{Papp (A to B)}}$$