CELL CULTURE-BASED MODELS FOR INTESTINAL PERMEABILITY

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Role of intestinal absorption assays in drug development.

Powerful methods have recently been developed for the combinatorial synthesis of organic compounds as have methods for high-throughput screening of pharmacological activity. As a result, large number of potential drug candidates are being obtained. This has increased the demand of screening methods for oral drug absorption during preclinical trials, suggesting an interest in cell culture models for experimental prediction of intestinal permeability.

Caco-2 cells.

Standard model for assessing the intestinal permeability and the most extensively characterized. However, its limitations must not be overlooked:
- Lack of correlation for active transported compounds due to underexpression of carrier proteins.
- Lack of correlation for paracellular absorbed compounds.
- Lack of drug metabolic enzymes such as isozyme (CYP)3A4.
- Cacophilicity: non-specific drug binding to Caco-2 cells, causing underestimation of permeability values.

Comparison of Caco-2 model and in vivo situation of typical drugs absorbed through different pathways:

| Squares | Transcellular route: 2-4 fold slower rate |
| Circles | Human jejunum |
| Naproxen | Circles |
| Atenolol | Paracellular route: 30- to 80-fold slower rate |
| L-dopa | Carrier-mediated: >100-fold slower rate |

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Utility of cell culture assays to measure drug absorption into the discovery paradigm of new pharmaceuticals.

TC-7 Cells

Obtained from cloning passage nº81 of Caco-2 parental cell line. Expression of many metabolic enzymes that are absent in Caco-2.
Non-suital model to predict absorption of paracellular and carrier-mediated compounds.
Does not represent a significant advantage respect Caco-2 cell line.

Transfected Cells

DNA transfection with genes from efflux transporters (MDR1 and MDR2 from Multidrug Resistance Protein family) increasing the predictability of the model.
Has not been implemented to high-throughput screening because it is not enough simple and reproducible as Caco-2 model.

Caco-2/HT29-MTX

Co-culture with HT29-MTX cell line, which is characterised to be mucin-producer model. Mimics better the in vivo situation than monolayers.
However, the role of mucus does not represent an important contribution to determine the permeability.

Three-Dimensional model

Co-culture with Caco-2 and HT29/MTX is indirectly seeded with collagen gel and stromal cells (immunocytes and fibroblasts).
Lower TEER values and increased permeability values provides an improved correlation with in vivo situation.

Conclusion.

Although intestinal absorption is a well known physiological process, a perfect model with mics perfectly its properties does not exist.
However, Caco-2 model can be used to identify drugs with potential absorption problems, and also to select drugs with optimal passive absorption characteristics from series of pharmacologically active compounds generated. Then, the principal challenge to reach an improved scenario is to optimize cell-based permeability assays by reducing costs and enable even greater applicability to drug discovery programs.

References.
[3] R. van Breemen and Y. Li, “Ideal for paracellularly absorbed compounds due to have leakier pores: pore radius is 9.0 ± 0.2 Å (similar to human small intestine), comparing to 3.6 ± 0.1 Å pore radius of Caco-2 cell line.

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Higher throughput: Optimization.

Several approaches have been persuaded to screen major number of compounds in a short period of time:
- Automation of cell culture phase.
- Miniaturization by using 96-transwell plates.
- N-in-one analysis: 5-10 co-incubated compounds are simultaneously analysed. Potential drug interactions can occur and should be taken into account.
- Shorter is better:
  - Use of improved culturing media and modified well plates (BD BioCoat™) → 3 days of Caco-2 culture to reach confluency.
  - Use of another cell line → 2/4/A1 reduces culture time and possesses better correlation than Caco-2 model.

Figure modified from [1].

Data and figure obtained and modified from [1].

Cell culture models for intestinal permeability.

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