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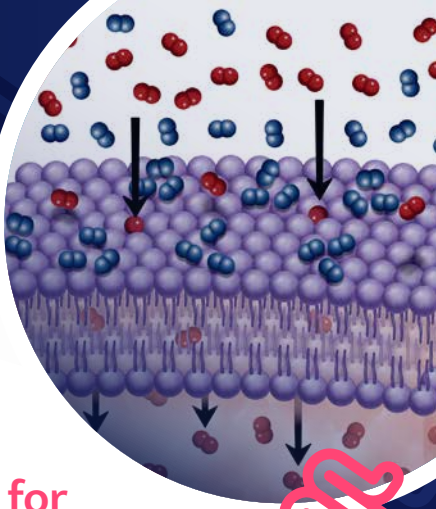
DDA Platform - Drug Discovery Accelerator

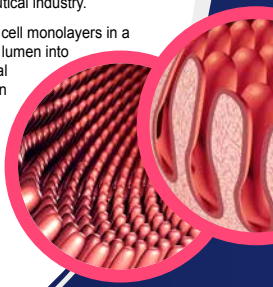


Predictive Models for Intestinal Drug Absorption

Intestinal drug absorption is one of the factors that determine the success of an oral drug product along with its efficacy, pharmacokinetics, and toxicity. Therefore, models for predicting intestinal drug absorption are becoming more important in early drug development to accelerate identification of promising or troublesome compounds.

Drug discovery scientists use many techniques to evaluate the intestinal permeability of drug candidates during the drug selection process. The most common preclinical methods currently used throughout the industry are: *in vitro* methods, for example, Ussing chamber or membrane vesicles based on animal tissue; cell-based assay systems such as Caco-2 cells and Mardin-Darby canine kidney (MDCK); artificial lipid-based systems such as parallel artificial membrane permeability assay (PAMPA) or immobilized artificial membranes (IAM); *in vivo* methods (animal pharmacokinetic studies); *in situ* methods (single-pass perfusion); and *in silico* (computer-aided drug design) methods. One, or a combination of these models, is commonly used in permeability assessment in drug discovery. A tiered approach is often used, which involves a high-throughput (but less predictive) model for primary screening, and then a low-throughput (but more predictive) model for secondary screening and mechanism study. The cell culture models strike the right balance between predictability and throughput, and are therefore the method of choice for permeability assessment across the pharmaceutical industry.

The use of cell cultures provides a method to predict drug permeability by utilizing cell monolayers in a two-chamber diffusion system to simulate the passage of drugs from the intestinal lumen into the blood. The cell model is simple and easy to use and avoids the usage of animal models for pharmacological and toxicological studies, so it is cost-effective and can produce reliable and reproducible results for understanding andevaluating the permeability characteristics of the potential lead drug candidates.



Many cell monolayer models have been developed to mimic human intestinal epithelium and are gaining in popularity, including Madin Darby canine kidney (MDCK), TC-7, HT29-MTX, 2/4/A1, and the most popular, Caco-2 cells. These models use immortalized cells that grow rapidly into confluent monolayers and undergo spontaneous differentiation, thus providing an ideal system for studying intestinal drug absorption.

Caco-2 cell represent a reference model for predicting drug permeability, and are routinely used to study transepithelial drug transport for the passive transcellular route, paracellular route, carrier-mediated route and transcytosis. Caco-2 cell lines, derived from human colorectal carcinoma, are cultivated on semipermeable filters for 21-23 days. After differentiation, the cells form a polarized monolayer with brush border, microvilli and tight junctions on the apical and basolateral sides, and express P-gp and several relevant efflux transporters and enzymes.

The Madin-Darby canine kidney (MDCK) is considered as an alternative to Caco- 2 cell for permeability studies. MDCK cells exhibit a shorter culture time (3-5 days) and lower transepithelial electrical resistance (TEER) values compared with Caco-2 cells (MDCK values are much closer to the *in vivo* TEER of the small intestine). However, these cells are derived from canine kidney and therefore the expression levels and metabolic activity of some transporters are quite different as compared to Caco-2 cells.

TC7, a subclone of Caco-2 cell, is also used for permeability screening. The TC7 shows morphological characteristics of brush border membrane, microvilli and tight junctions similar to Caco-2 monolayer. Therefore, the TC7 model offers an alternative to Caco-2 to evaluate the intestinal permeability of test compounds. In addition, TC7 has an advantage over Caco-2 by expressing high levels of CYP3A4 enzymes well represented in the intestine. However, TC7 lacks transport proteins, so its application is biased towards drug metabolized by CYP3A4.

The human adenocarcinoma HT29-MTX model is used to study the role of intestinal mucus on drug absorption across the intestinal barrier. HT29-MTX is conditioned to acquire the morphological and mucin producing features of goblet cells by culturing parental HT29 in a medium containing methotrexate (MTX) for 6 months. Unlike Caco-2, HT29-MTX develops sparse microvilli on the apical side and reaches confluence 3 days later than the former. However, the expression of goblet cells in HT29-MTX increases absorption of lipophilic compounds compared to Caco-2 monolayer.

2/4/A1 originates from the intestine of fetal rat and is believed to mimic intestinal passive paracellular permeability in humans better than Caco-2 monolayer. This immortalized cell is reported to differentiate into a monolayer with tight junctions, brush-border membrane enzymes and transporter proteins. Unlike Caco-2, the tight junctions expressed in 2/4/A1 are loose and better for studying compounds absorbed in the human intestine through the paracellular route.

IEC-18 cell line, also of rat origin, derived from native ileal crypts and is a valuable model for studying the permeability and paracellular transport across intestinal epithelium. These cells are also used to study the effects of enzymes and receptors on the permeability of drugs. Nevertheless, because they are less well differentiated than Caco-2 cells, some of the carrier-mediated transport is absent.

LLC-PK1 cells derived from pig kidney epithelium have also been employed as an alternative model to Caco-2 cells to assess the permeability of test compounds. Studies have reported the utility of LLC-PK1 in characterizing the passive (transcellular and paracellular) absorption of test compounds.

T84 is a cell line derived from human colon carcinoma, which spontaneously differentiates to form polarized monolayer with well-formed tight junctions. T84 cells are similar to adult colonic crypt cells in morphology, tight junctions, and ion transport characteristics. Unlike Caco-2 cells, T84 cells are less prone to differentiate into sublines with altered characteristics.

Although all of these cell models show good or moderate correlations with passively absorbed drug permeability in humans, correlations with active transport are variable and mainly low. However, even if *in vivo* correlation is slow, they are interesting models for determining drug transport mechanism, and extensive studies are needed to identify the relevant carriers and active transport mechanisms.

Table 1. Comparison of models for predicting intestinal absorption.

	Characteristics	Pros	Cons
In Silico	✓ Computational model analysis based on the physicochemical properties of compounds ✓ Quantitative structure-activity relationship (QSAR) model ✓ Artificial neural networks	✓ Quick and inexpensive method for assessing intestinal permeability	✓ Not as reliable as real experimental data
In Vitro	✓ Cultured cell monolayer: widely used in absorption studies	✓ Less labor ✓ Less cost-intensive ✓ Benefits in terms of ethical considerations	✓ Failure to consider the effect of physiological factors ✓ Interlaboratory variation interferes with the extrapolation of *in vitro* transport data
In Situ	✓ Perfusion of drug through isolated intestinal segment	✓ Intact blood and nerve supply ✓ Appears to correlate best with the human data	✓ Massive animal consumption ✓ High amounts of test compounds ✓ Not sensitive enough to measure compounds with low or moderate permeability
Ex Vivo	✓ Excised animal tissues mounted on Ussing chambers	✓ Drug transport can be investigated ✓ Amount of drug needed is relatively small	✓ Time consuming ✓ Difficult to perform epithelial tissue dissection
In Vivo	✓ Commonly used animals include rats, monkeys, dogs and pigs, of which rats are the most frequently used	✓ Integrate all dynamic components that may affect drug dissolution	✓ Massive animal consumption ✓ Time-consuming ✓ Labor-intensive nature ✓ Impossible to separate the variables involved in the process of absorption

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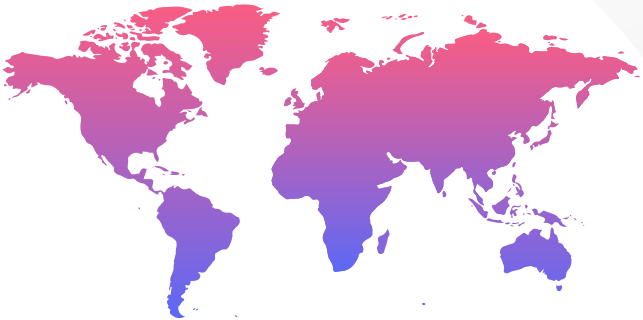

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