IN VITRO GASTROINTESTINAL DRUG TRANSPORT PREDICTION BY CACO-2, MDCK AND PAMPA MODELS: AN OVERVIEW

Abstract

Majority of drug candidates fail at phase II and phase III clinical trials due to in appropriate pharmacokinetic parameter. Recently emerge in vitro model such as Cacco-2, MDCK and PAMPA can compute drug intestinal permeability. If drug's absorption and permeability profile found unsuitable it can be eliminated from trial. Some modification in Caco2 model enables Caco2 that closely resembles with in vivo intestinal mucosa. Integrity of tight junction dynamics in cell culture Caco2 models of endothelial and epithelial monolayers can be measured by Transepithelial /transendothelial electrical resistance. Goblet cells are mucus-producing cells condition has been be developed by incorporating human colon adenocarcinoma mucus-secreting cells. Caco2 communication with other cells of native intestine has been developed by introducing humanized three-dimensional (3D) co-culture grown on the surface of a subepithelial-like tissue construction containing intestinal or dermal fibroblasts. These MDR1-MDCK cells originate from transfection of Madin Darby canine kidney (MDCK) cells with the MDR1 gene, encoding for the efflux protein transporter P-glycoprotein. Permeability assay is a valuable tool for the identification and characterization of P-gp substrates and inhibitors. PAMPA sandwich model where one plate contains a porous filter disk at the bottom of each well and another is a reservoir plate in between the plate filter which contains lipid layer placed to simulate the artificial membrane. These in vitro models has significantly reduces the time and cost of drug discovery.

Keywords: Caco-2, MDCK and PAMPA, Absorption, Permeability.

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I. INTRODUCTION

Historically, drug discovery has focused on efficacy and selectivity against the biological target such as receptor, enzyme, ion channel transport etc. However not much attention was given for drug pharmacokinetics properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET). Due to ignorance these crucial parameter; majority of drug candidates fail at phase II and phase III clinical trials. Preclinical and post clinical evaluation of pharmacokinetic is a time consuming process and required of new chemical entity requires lot of time, skills, acquisitive cost. The pharmaceutical industry are under huge pressure to reduce or control the escalating cost of new drug development which has led to investigation of in-vitro model to predict drug transport through gastro-intestinal tract. [Duxin Sun et al, 2022] Caco-2 and MDCK cell monolayers are widely used to simulate membrane permeability as an in vitro estimation of in vivo absorption. These in vitro findings allowed for the development of in silico models that might be used to predict the ADMET characteristics of drugs even before they are manufactured.

Intestinal permeability of drug remains as a critical factor for design, optimization, and selection of drugs and pharmaceutical formulations for oral delivery. Effective permeability value (P_{eff}) refers to human jejunal permeability. In the absence of the measured value of preclinical assessment, an estimated value derived from in silico prediction (ADMET Predictor), in vitro measurements (e.g. CaCo2, PAMPA test), or animal studies (rat, dog) can be employed in the simulation. For this reason, the application includes a permeability converter that converts the given input value to human P eff using a correlation model created from a training data set. [Sandra Grbic et al 2013]. Input data of in vitro permeability models, solubility measurement and dissolution characteristics in different media gives accurate prediction of absorption profile of investigational drugs. [Hugo Almeida et al 2022]

In vitro models are essential tools in pharmaceutical research for predicting various aspects of drug behaviour, including drug transport across biological barriers like the gastrointestinal (GI) tract. Three commonly used in vitro models for predicting gastrointestinal drug transport are Caco-2, MDCK, and PAMPA models. Here's an overview of each model and how they are used for drug transport prediction:

1. **Caco-2 Model:** Caco-2 cells are human epithelial cells derived from a colon carcinoma. These cells spontaneously differentiate in culture to form monolayers with tight junctions, resembling the intestinal epithelium. The Caco-2 model is widely used to study drug transport across the intestinal epithelium and predict drug absorption. The Caco-2 model provides insights into various aspects of drug transport, including passive diffusion, active transport, and efflux mechanisms. Researchers can measure drug permeability, assess interactions with transporters, and predict the potential for oral drug absorption. It's a valuable tool for early drug development, as it helps identify compounds with good oral bioavailability and those that might face absorption challenges

2. **MDCK Model:** MDCK (Madin-Darby Canine Kidney) cells are derived from dog kidney tissue and are often used as a model for studying drug transport across various epithelial barriers, including the intestinal barrier. Like the Caco-2 model, MDCK cells can form tight monolayers that mimic epithelial barriers.

The MDCK model is particularly useful for studying the transport of compounds that might be substrates for P-glycoprotein (P-gp) and other efflux transporters. It's also used to assess drug permeability and interactions with transporters in a similar manner to the Caco-2 model.

3. **PAMPA Model:** PAMPA stands for Parallel Artificial Membrane Permeability Assay. This model involves creating an artificial lipid bilayer barrier that mimics cell membranes. It's a high-throughput method used to predict passive drug permeability based on the compound's ability to partition between an aqueous and a lipid layer.

The PAMPA model is useful for quickly assessing the passive permeability of a large number of compounds. It doesn't account for active transport or transporter interactions, making it more suitable for preliminary assessments of drug permeability. Each of these models has its strengths and limitations. Caco-2 and MDCK models provide more physiological relevance and can account for active transport processes, while the PAMPA model is faster and more high-throughput. Researchers often use a combination of these models to gain a comprehensive understanding of a drug's transport behaviour across the GI tract and other barriers. It's important to note that while these in vitro models provide valuable insights, they are simplifications of complex biological processes. In vivo studies, such as pharmacokinetic studies in animals and clinical trials in humans, are necessary to validate and refine the predictions made by these models.

II. CACO-2 MODEL

1. **Monolayer Formulation:** Caco-2 cells are cultured on permeable supports to form monolayers that mimic the intestinal epithelium. As these cells differentiate, they develop tight junctions, creating a barrier that separates the apical (luminal) and basolateral (blood) sides, resembling the physiological conditions of the GI tract.

2. **Measurement of Permeability:** primary application of the Caco-2 model is to assess drug permeability across this monolayer. Permeability studies are performed by introducing the drug of interest to the apical side of the monolayer and measuring its appearance on the basolateral side over time. This provides insight into the drug's ability to traverse the intestinal epithelium and its potential for absorption.

3. **Assessment of Absorption Mechanisms:** Caco-2 monolayers allow researchers to study both passive and active mechanisms of drug transport. Passive diffusion is the movement of a drug across the membrane driven by concentration gradients, while active transport involves the participation of transporters that can pump the drug against its concentration gradient. By measuring drug transport in both directions (A-B and B-A), researchers can infer the involvement of active efflux and uptake mechanisms.

4. **Efflux Transporters and Drug Interactions:** Caco-2 cells express efflux transporters like P-glycoprotein (P-gp) and others, which play a significant role in limiting the absorption of certain drugs. By inhibiting these transporters with specific inhibitors, researchers can evaluate the potential impact of efflux on the drug's absorption. Additionally, co-incubation with other drugs can help predict drug-drug interactions, which might affect absorption.

5. **Permeability Classification and Bioavailability Prediction:** Based on the permeability data generated from Caco-2 experiments, drugs are often categorized into classes, such as high-permeability and low-permeability compounds. These classifications assist in predicting the fraction of orally administered drugs that will be absorbed into the systemic circulation, thus influencing bioavailability predictions.

6. **Transporter Substrate and Inhibitor Studies:** Caco-2 studies can determine whether a drug is a substrate for specific transporters by observing changes in its transport when transporters are inhibited or if the drug is structurally similar to known transporter substrates. This information aids in understanding the potential for transporter-mediated interactions and absorption.

7. **Challenges and Limitations:** While the Caco-2 model offers valuable insights, it does have limitations. It represents a simplified model of the complex GI tract and lacks the dynamic environment of blood flow, enzymes, and other factors present in vivo. Thus, while the model provides a good initial prediction of GI drug transport, it's important to validate results in more complex systems and in vivo studies.

The Caco-2 model is a fundamental tool in the pharmaceutical industry for predicting the transport and absorption behavior of drugs in the gastrointestinal tract. It offers valuable insights into passive and active transport mechanisms, drug-drug interactions, and overall absorption potential, aiding in the decision-making process during drug development.

III. MDCK (MADIN-DARBY CANINE KIDNEY) MODEL

1. **Epithelial Monolayer Formulation:** Similar to the Caco-2 model, the MDCK model involves culturing cells to form a monolayer that simulates the intestinal epithelium. MDCK cells, originally derived from a dog's kidney tissue, are used to create these monolayers.

2. **Drug Permeability Assessment:** The primary purpose of the MDCK model in GI drug transport prediction is to measure drug permeability across the monolayer. Permeability studies are conducted by exposing the monolayer to the drug and observing its movement from the apical to the basolateral side and vice versa.

3. **Efflux Transport and P-Glycoprotein (P-gp):** MDCK cells also express efflux transporters like P-gp. This allows researchers to study the impact of efflux mechanisms on drug absorption. Similar to the Caco-2 model, the MDCK model can be used to assess whether a drug is a substrate for P-gp and other efflux transporters.

4. **Comparison with Caco-2 Model:** While both the Caco-2 and MDCK models are used to predict GI drug transport, they have differences. The MDCK model is often used when a specific focus is on transporter interactions and P-gp-mediated efflux, as these cells have a lower expression of these transporters compared to Caco-2 cells.

5. **Drug Drug Interactions and Transporter Substrates:** Researchers can use the MDCK model to evaluate drug-drug interactions related to transporters. Inhibition studies with

known transporter inhibitors can help predict how co-administered drugs might affect each other's absorption due to competition for transporter-mediated transport.

6. **Challenges and Limitations:** Similar to the Caco-2 model, the MDCK model has its limitations. It's a simplified representation of the complex intestinal environment and lacks the full complement of human transporters and metabolic enzymes. Therefore, the results obtained from the MDCK model should be interpreted cautiously and ideally validated in more complex systems or in vivo studies.

The MDCK model, like the Caco-2 model, provides a way to assess the transport and permeability of drugs across a monolayer that simulates the intestinal epithelium. It's particularly useful for studying P-gp-mediated efflux and transporter interactions. However, it's important to remember that while these in vitro models are valuable tools in early drug development, they are not perfect substitutes for in vivo studies and should be used in conjunction with other methods for a comprehensive understanding of drug transport behavior.

IV. PAMPA MODEL

The PAMPA model is an in vitro technique used to predict the passive permeability of drugs and other compounds across biological membranes, including those found in the gastrointestinal tract. It's a high-throughput method that involves creating an artificial lipid membrane barrier that simulates the lipid bilayer of cell membranes. This model is particularly useful for assessing passive transport, and it's often used as a screening tool during early stages of drug development. The PMPA model works as follows:

1. **Artificial Membrane Setup:** In the PAMPA model, a donor compartment contains the compound of interest in a suitable solvent, while an acceptor compartment contains a buffer solution. A lipid solution is used to create an artificial lipid bilayer that mimics cell membranes. The lipid bilayer is immobilized between the donor and acceptor compartments

2. **Passive Diffusion Measurement:** The compound is introduced into the donor compartment, and over time, it will partition between the donor solution and the artificial lipid membrane. If the compound has good lipid solubility, it will readily diffuse across the lipid bilayer into the acceptor compartment. The rate of diffusion is measured, often using techniques like UV spectroscopy or liquid chromatography, to determine the compound's permeability coefficient.

3. **Permeability Predictions:** The permeability coefficient obtained from the PAMPA assay is used to predict the compound's passive permeability. Higher permeability coefficients suggest that a compound is more likely to passively diffuse through biological membranes, including those in the GI tract.

4. **Advantages and Limitations:** The PAMPA model is advantageous due to its simplicity, high throughput, and ability to assess passive permeability quickly. It provides a way to screen a large number of compounds and identify candidates with potential for good absorption. However, it's important to note that the PAMPA model is a simplified

representation of the complex biological processes that occur in vivo. It doesn't consider active transport, efflux pumps, or interactions with transporters and enzymes that are present in living organisms. As such, while the PAMPA model can provide insights into passive permeability, it should be used in conjunction with other models, such as the Caco-2 and MDCK models, for a more comprehensive understanding of drug transport and absorption behavior in the GI tract. In summary, the PAMPA model is a valuable tool for predicting passive permeability and identifying compounds with potential for absorption across biological membranes, including the gastrointestinal epithelium. It is especially useful in the early stages of drug development for screening a large number of compounds quickly.

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