In vitro models to determine the pharmacokinetic parameters

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ABSTRACT

The pharmaceutical industry seeks to more efficient and precise determination of the main pharmacokinetic parameters of existing and new drugs, and therefore uses new in vitro models that show a high degree of correlation with in vivo response. Determination of intestinal absorption, metabolic transformations and metabolic profile of drugs is carried out using the laboratory in vitro models, which represent innovative basis for determination of absorption, distribution, metabolism and elimination (ADME) of drugs.

The aim of this paper is to present three most important in vitro models, their properties and applications in the pharmaceutical industry when determining the pharmacokinetic parameters.

The rapid development of the pharmaceutical industry expressed the need for in vitro models for the determination of pharmacokinetic parameters in the laboratories that want to increase their operational efficiency and effectiveness.

INTRODUCTION

The prediction of human pharmacokinetic and disposition attributes of new drugs from preclinical data has become a mainstay of drug metabolism and pharmacokinetics organizations within pharmaceutical research and development operations. In the vast majority of large research and development groups, the nomination of new compounds into the development phase requires a prediction of what the human pharmacokinetics will be.

Models have been developed to assess important human disposition attributes during the drug design phase so that scientist can simultaneously optimize absorption, distribution, metabolism, and excretion properties and pharmacological potency. These models and methods are standard in modern drug discovery, and are a critical element to successful drug discovery[1].

The majority of drugs available on the market are in oral dosage forms. For the assessment of permeability and oral drug absorption and metabolism, in vitro and high throughput systems, such as the parallel artificial membrane permeability assay (PAMPA) and cell-based systems exist to relate drug permeability to absorption, especially for compounds that do not undergo intestinal metabolism.

The most popular high-throughput screening tool for drug permeability is human colon carcinoma (Caco-2) or transfected Madin-Darby canine kidney (MDCK) cells [2].

IN VITRO MODELS TO DETERMINE THE PHARMACOKINETIC PARAMETERS

Caco-2 cell lines

A popular in vitro model is the Caco-2 cell line, derived from human colon carcinoma cells that contains the P-glycoprotein (Pgp). Studying the permeability of
compounds across a Caco-2 cell monolayer is an established in vitro model to screen for oral absorption and to evaluate the mechanism of transport.

In culture, they differentiate spontaneously into polarized intestinal cells possessing an apical brush border and tight junctions between adjacent cells, and they express hydrolases and typical microvillar transporters. This cell line was first used as a model for studying differentiation in the intestinal epithelium, and later for estimating the relative contributions of paracellular and transcellular passage in drug absorption. Caco-2 cells, despite their colonic origin, express in culture the majority of the morphological and functional characteristics of small intestinal absorptive cells, including phase I and phase II enzymes, detected either by measurement of their activities toward specific substrates, or by immunological techniques [1].

The Caco-2 permeability model is also considered to be the industry reference standard for in vitro prediction of in vivo human intestinal permeability, bioavailability and drug-drug interactions (DDIs) of orally administered drugs.

The development of the Caco-2 cell has greatly facilitated progress and led to the testing of diverse drug classes as P-gp. More recent development involved transfection with the cytochrome P450 gene and stimulation by butyrate to provide the added P450 activity. The incubation system, the donor and receiving compartments separated by the cell monolayer, is an efficient, high-throughput system for examination of whether newly developed pharmaceuticals are substrates of cytochrome P450 3A4 and/or P-glycoprotein such that interactions with other drugs may be predicted [2].

Most studies with Caco-2 monolayers were performed to determine whether a drug is actively or passively transported across the intestinal epithelium, and to provide new insights into the regulation of drug transport. So Caco-2 model has become the gold standard to relate drug permeability to oral drug absorption [3].

**Parallel artificial membrane permeability assay**

Parallel artificial membrane permeability assay is a model which determines the permeability of substances from a donor compartment, through a lipid-infused artificial membrane into an acceptor compartment. A multi-well microtitre plate is used for the donor and a membrane/acceptor compartment is placed on top; the whole assembly is commonly referred to as a “sandwich”. At the beginning of the test, the drug is added to the donor compartment, and the acceptor compartment is drug-free. After an incubation period which may include stirring, the sandwich is separated and the amount of drug is measured in each compartment. Mass balance allows calculation of drug that remains in the membrane. To date, PAMPA models have been developed that exhibit a high degree of correlation with permeation across a variety of barriers, including Caco-2 cultures, the gastrointestinal tract, blood–brain barrier and skin. The donor and/or acceptor compartments may contain solubilizing agents, or additives that bind the drugs as they permeate. To improve the in vitro - in vivo correlation and performance of the PAMPA method, the lipid, pH and chemical composition of the system is often designed with biomimetic considerations in mind.

Furthermore, PAMPA only measures permeability by passive diffusion whereas the Caco-2 permeability assay also assesses active uptake/efflux and paracellular transport. Therefore, a good correlation is observed between the Caco-2 permeability assay and PAMPA if the compound crosses the membrane by passive diffusion alone.

If the compound is a substrate for active efflux then the PAMPA overestimates the permeability and if the compound undergoes active uptake or paracellular then the PAMPA underestimates the permeability [4,5].

**Madin-Darby Canine Kidney cells**

Apart from Caco-2 cells other models that are most frequently used for ADME studies are MDCK cells. When the MDCK cells are cultured under standard conditions, they differentiate into polarized columnar epithelial cells and form tight cellular junction. The main advantage of MDCK cells is shorter culture time, which can be equal to 24 hours. A good correlation was reported between permeation of passively absorbed drugs in Caco-2 and MDCK cells. The permeability coefficients of hydrophilic compounds are usually lower in Caco-2 cells than in MDCK cells. Whereas Caco-2 cells originate from human colon adenocarcinoma cells, MDCK cells are from dog kidney cells, and thus the expression levels of intestinal transporters would be different in these two cell lines [6].

**Conclusion**

In vitro models can be used to study the ADME properties of drugs or general chemicals. Caco-2 cell lines can be performed to estimate the absorption of compounds through the lining of the gastrointestinal tract. The versatility of Caco-2 cells is demonstrated by the fact that, even to this day, they are serving as the basis for the creation of innovative new models that are contributing to our understanding of drug efflux transporters such as P-glycoprotein.

As financial and other factors in pharmaceutical development continue to strain timelines and resources, PAMPA will most likely continue to play an ever-increasing role in laboratories wishing to increase their operating efficiencies and success rate.
Declaration of interest
The authors declare no conflict of interest for this study.

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