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Review on Drug Absorption Studies

Vimalavathini R*, Subhashri SHR, Kavimani S
Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health sciences, Puducherry.

ABSTRACT

Drug absorption is an important criteria for pharmacokinetic and pharmacodynamics action of drug. Various approaches to evaluate drug absorption potential include in silico models, in vitro models, ex-vivo models, in situ and in vivo models. The procedures, advantages, disadvantages for various screening models are discussed in this review.

Keywords: Absorption, permeability, ex-vivo, in-situ, in-vivo

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INTRODUCTION

Subsequent to oral drug administration, there are several possible sites of drug loss such as metabolism, erratic dissolution and altered gastrointestinal permeability. Hence, absorption and metabolism of drug at the intestine is crucial because it regulates the bioavailability of these drugs. [1, 2] Absorption models can be studied by *in silico, in-vitro, ex-vivo, in-situ* and *in-vivo models*. The choice of models depends on the properties of the test compound being studied.

A. IN SILICO PREDICTION OF ABSORPTION

Lipinski's rule of five is the best-known computational models to predict intestinal absorption based on molecular properties. However, it does not apply to compounds that are subject to active transport processes. Another disadvantage of this model is that it is mainly built for compounds with a high absorption (Hilde Bohets et al). QMPRPlus™, GastroPlus™ and iDEA™, DDDPlus, GastroPlus, MapCheck are examples of such absorption study software packages available. [2, 3]

B. IN-VITRO MODELS

In vitro study models include caco-2 cell lines and Madin Darby canine kidney (MDCK) cells, transcellular transport studies in cell monolayers and assays such as parallel artificial membrane permeability assay (PAMPA), p-gp ATPase assay, calcein-AM P-gp inhibition assay, isolated membrane vesicles and chromatographic retention indices. To measure transport across membranes and studying the effect of active processes transcellular transport assays are performed in both apical to basolateral and basolateral to apical directions.

1. Caco-2 cell culture

Caco-2 cell culture is widely used as a tissue model for studying bioavailability and transport processes, adhesion of probiotic bacteria or pathogens and immune responses following allergen invasion. With this passive as well as active transport can be studied. The main advantage of the Caco-2 cells is that they are of human intestinal origin (i.e) they are derived from human colon adenocarcinoma. They form tight junctions, spontaneously differentiate, express relatively high levels of digestive brush border enzymes and display other, structural and functional properties similar to intestinal enterocytes. They express most receptors, transporters and drug metabolizing enzymes like amino peptidase, esterase and sulfatase found in normal epithelium. However, there is no P-450 metabolizing enzyme activity and lack of other cell types leading to deficiency of absorption of bile salts and phospholipids.[4] Caco-2 cells are grown in transwell plates and after tight junctions have formed in the monolayer. The trans-epithelial electrical resistance (TEER) of the monolayer in each well is measured after incubation of cells with or without drug in the transport buffer.

2. Madin Darby canine kidney cells

MDCK cells were isolated from a dog kidney are currently used to study the regulation of cell growth, drug metabolism, toxicity and transport at the distal renal tubule epithelial level. They differentiate into columnar epithelial cells and form tight junctions and monolayer of polarized cells within 3-5 days when compared to Caco-2 cells MDCK cells. They also exhibit TEER values which are more comparable with the TEER values of the small intestine *in vivo*. However, the MDCK cells are of canine origin, it is important to correct for any underlying canine transporter activity.

3. Parallel artificial membrane permeability assay (artificial membrane)

PAMPA is useful for high throughput screening and to study the permeability pH profiles or the influence of the unstirred water layer on the permeability. PAMPA data can help permeation assessment of a metabolically unstable compound. A combination of PAMPA and a high-throughput solubility assay enables biopharmaceutical classification in early drug discovery. It provides information on the solubility, lipophilicity and ionization status and permeability properties of a drug. The main disadvantage is that they lack active transport process. Also in PAMPA the membrane is not the biological membrane. [2]

4. P-gp ATPase assay

Adenosine triphosphate binding cassette (ABC) transporters pump substrates out of the cell by using ATP (adenosine triphosphate) hydrolysis as an energy source. ATP hydrolysis yields inorganic phosphate (Pi) and the amount of Pi liberated by the transporter is proportional to the activity of the transporter. ATPase activity can be estimated by quantifying ATP, ADP [5] released NADP, or by quantifying liberated inorganic phosphate. ATPase activity can be determined using cell membranes, cultured cells and membrane vesicles. Since ATPase assay indirectly estimates efflux transport and does not provide the kinetics of drug transport and/or inhibition. [6,7]

5. Calcein-AM P-gp Inhibition Assay

The calcein-AM assay is based on calcein-acetoxymethylester (calcein-AM), a non-fluorescent p-glycoprotein substrate, being hydrolyzed inside the cell to calcein. Calcein is a fluorescent molecule that is trapped inside the cell and can be detected by fluorometric analysis. Intracellular fluorescence is evaluated as a measure of p-glycoprotein interaction. Cells such as porcine brain capillary endothelial cells are used. The advantage of these methods is the ease of detection and the possibility for high throughput analysis. [8]

6. Isolated membrane vesicles

Membrane vesicles from intestinal scrapings or isolated enterocytes are being extensively used in transport characterization studies. The brush border membrane vesicles prepared from

intestinal tissues provide the flexibility of examining the interaction of drugs to a specific membrane of interest (e.g. brush border membrane vs. basolateral membrane of enterocytes). They are used to study the properties of drugs, nutrient transport at cellular level and allow a complete manipulation of solute environment both inside and outside of the vesicle. The process of isolation of vesicles often leads to damage of the transporter proteins and enzymes and a sensitive analytical method is required for this method of permeability study, since the volume of the vesicles is extremely small.

7. Chromatographic retention indices

Immobilized artificial membrane (IAM) packings are prepared by covalently immobilizing monolayer of cell membrane phospholipids to silica particles. These IAM surfaces mimic fluid cell membranes. IAM and micellar liquid chromatography (MLC) are used to evaluate the passive intestinal absorption of drugs. An important advantage of the chromatographic techniques is ease of operation and a high analytical sensitivity. However, compounds with low permeability pose problem. [9]

C. EX-VIVO MODELS

Ex-vivo techniques can be performed using Everted sac model and Ussing chambers.

1. Everted sac model

Everted sac model can be performed in two ways the everted sac model and non-everted sac model. This kind of model is suitable for measuring absorption at different sites in the small intestine for estimating the first-pass metabolism of drugs in intestinal epithelial cells and to study the effect of P-gp on xenobiotic transport through the intestinal barrier. [10]

The everted gut sac of the rat small intestine can be used to determine kinetic parameters with high reliability and reproducibility. It is simple, cheap and rapid model to perform. Major drawback of this method is the presence of the muscularis mucosa; hence the drugs have to pass through the muscularis mucosa. [11]

2. Ussing chambers

The Ussing chamber is an instrument in which human or animal intestines or mucous membranes are fixed between a receiving pool and a diffusion pool containing the drugs. After a period of incubation, the drug concentrations on both sides of the membrane are measured to determine the rate of drug absorption. Advantages are the activity of the small intestine can be estimated by determining the resistance of the intestinal membrane, the absorption of different segments of intestine can be studied, and the test samples are clean and easy to be analyzed. Disadvantages include lack of blood and nerve supply, which results in vulnerability of the mucosa, rapid loss of mucosa activity, and relatively low-transport during the procedure. Ussing chambers are useful for studying the effects of compounds on

electro-physiological parameters of the intestinal barrier, ion transport across many types of membrane and intestinal metabolism of xenobiotics. [12,13]

D. IN-SITU INTESTINAL PERFUSION TECHNIQUE

In-situ technique includes intestinal perfusion with venous sampling models, the isolated and vascular perfused intestinal models, mesenteric lymph duct cannulated anaesthetized rat model, anaesthetized large animal model. The main advantage is that the technique produces absorption rates which are realistic and comparable to data obtained by oral drug administration. The experimental technique is simple, reliable and inexpensive. Multiple samples may be taken, and performed many kinetic studies. Animal to animal variation of the kinetic results is minimal, and the time required to conduct an experiment is moderate. Also the rat model has been demonstrated to correlate with in vivo human data. Although, the animal has been anaesthetized and surgically manipulated, neural, endocrine, lymphatic, and mesenteric blood supplies are intact. Absorption at particular region of the intestine can be studied. Direct effects of the drug on intestinal absorption and secretion of substances into intestinal lumen by P-gp also can be studied. Its drawback includes surgical manipulation and anaesthetization of animals can adversely affect physiological factors that can alter drug absorption characteristics. It is not suitable for high throughput screening purposes. The rise in luminal hydrostatic pressure during absorption studies at particular sites can influence intestinal permeability. [14,21]

1. Intestinal perfusion technique

Intestinal perfusion technique can be performed in two different ways Closed loop intestinal perfusion technique and Open loop intestinal perfusion technique. In closed loop perfusion technique, a drug solution is placed in an isolated segment of the intestine and the resultant luminal solution is analyzed at different time points. On the other hand, open loop perfusion technique a continuous fluid flow is maintained down the intestine, and intestinal permeability is estimated through the concentration difference in inlet and outlet perfusate at steady state. Also the volume of the luminal drug solution as the water absorption and secretion during the perfusion may cause errors in the luminal concentrations and therefore in the calculated absorption rate. So, various water flux correction methods such as co-perfusion of a non-absorbed marker such as phenol red or inulin, or use the simpler gravimetric method are used. [15,21]

2. Intestinal perfusion with venous sampling models

Also referred as ‘auto-perfused’ method involves cannulation and drainage of a vein mainly mesenteric vein. The major advantage of this experimental technique therefore is that it facilitates both a quantification of steady stated is appearance kinetics from the intestinal

lumen and concurrent appearance kinetics in pre-hepatic blood. However, the limitation is that the drug absorption is predicted by quantifying net drug uptake into enterocyte cells and not net flux through the cell. [21]

3. The isolated and vascularly perfused intestinal models

These techniques involve isolating and cannulation of major artery of intestine (mesenteric artery) and drainage vein (mesenteric vein) of the intestine. The technique provides the best simulation of the in vivo situation. [16]

4. Mesenteric lymph duct cannulated anaesthetized rat model

Gastrointestinal absorption may occur via two principle routes: portal uptake or transport through the intestinal lymphatics. The intestinal lymphatics are responsible for the gastrointestinal uptake of dietary lipid and lipophilic nutrients and also lipophilic drugs. The most frequent of which involves a triple cannulation technique, involving cannulation of the carotid artery for the collection of systemic blood samples, the mesenteric lymph duct for the collection of the mesenteric lymph, and the duodenum for the administration of drug. The technique is useful in assessing intestinal versus hepatic first-pass metabolism in a controlled experimental setting. However, the major drawbacks are humoral/neurogenic removal on tissue viability and the highly specialized surgical procedures involved. [17,21]

5. Anaesthetized large animal model

Pig model involves jejunal single pass perfusion, with blood sampling from both portal vein and superior cava vein, in addition to bile duct collection. The combined perfusion and hepatobiliary sampling method was a useful method to quantitatively compare intestinal versus hepatic versus hepatobiliary-mediated elimination of the drug. [18,21]

E. IN-VIVO MODELS

In vivo techniques in animal models are cannulated conscious rat models, cannulated conscious large animal model, single-pass perfusion in conscious dog/pig, single-pass perfusion in conscious humans, in vivo Studies with portal vein sampling. The most frequently used animal model is the rat, since it better reflects the human situation with respect to paracellular space and metabolism. The advantages are in vivo models can integrate the dynamic components of the mucous layer, the mesenteric blood circulation and all other factors that can alter drug dissolution and Cassette dosing studies can give better information on drug bioavailability, including intestinal barrier passage be performed. This technique is useful to test large number of products. [19,20]

Large animal models such as dog, pig, humans allow for oral administration of clinically relevant human dosage forms and also gastric transit and biliary secretion profiles more closely resemble human. In addition cannulation of primary absorption routes (e.g. portal

vein, thoracic lymph duct) affords a unique mechanistic assessment of the drug absorption/elimination processes, which is not feasible and/or possible with smaller animal models or human studies. However these models are expensive to set up and monitor with ethical issues. There are a number of isolated intestinal perfusion techniques reported in humans, including open, semi-open, or closed perfusion of an intestinal segment, perfusion of a tied loop during surgery, colorectal perfusion, and colonic load via endoscope. [21]

CONCLUSION

This review focused on the recent progress in screening approaches for drug absorption with their potential advantages and disadvantages of each model being analyzed. Screening models such as *in silico, in vitro, ex vivo, in situ* are becoming more popular for prediction of drug metabolism and bioavailability. They allow reduction of use of animals in preliminary tests and being less complex they are easier to interpret and correlate with *in vivo* observations.

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