

REVIEW ARTICLE

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Evaluation of drug permeability methods recommended by health authorities

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Abstract

The oral route is the most preferred route for drug administration because of patient compliance. Following oral administration, a drug has to cross several biological barriers before reaching the systemic circulation by transcellular or paracellular transports. Oral drug absorption and bioavailability are influenced by the physicochemical, formulation and physiological variables. Oral absorption of drugs is controlled by dissolution from the dosage form, and permeability across the gastrointestinal membrane. Rate limiting step in absorption is the permeability for compounds with low permeability, and dissolution for compounds with low solubility. According to Food and Drug Administration (FDA) Biopharmaceutics Classification System (BCS)-based biowaiver guidance and European Medicines Agency (EMA) Guideline on the Investigation of Bioequivalence, permeability class of a drug substance can be determined using different methods. The aim of this study was to evaluate the methods in detail recommended by FDA and EMA for determination of permeability of drugs. We surveyed the literature as to the pros and cons of the methods recommended by the health authorities for assessment of permeability. In general, a single method is sufficient for determination of permeability. When a single method fails to demonstrate a permeability classification, two different methods may be used.

Keywords: Permeability, Food and Drug Administration (FDA), Biopharmaceutics Classification System (BCS), oral absorption, European Medicines Agency (EMA)

Introduction

Dosage forms are administered by various routes such as oral, buccal, rectal, sublingual, topical, transdermal, inhaler, vaginal, ophthalmic, intradermal, intramuscular, intravenous [1]. Among these routes, the oral route is the most preferred route for drug administration [2] because it is painless, safe, and cheaper than the other routes, and is self-administered by the patients. When a drug administered as a solid dosage form (e.g. tablets, capsules) by oral route, initially, it disintegrates into solid particles, and then these particles dissolve in the gastrointestinal fluids, and finally permeate from the gastrointestinal tract into systemic circulation [3, 4].

Drug absorption, and hence bioavailability are influenced by the physicochemical (e.g. particle size, salt forms, crystal forms, polymorphism, pH, pKa, complexation), formulation (e.g. process

parameters, excipients, preparation methods) and physiological (e.g. intestinal motility, gastric emptying, gastric flow rate, absorption surface area, interindividual differences) parameters [5].

Following oral administration, a drug has to cross several biological barriers before reaching the systemic circulation. Transport across the cell membranes are classified as transcellular and paracellular transports [6]. The transcellular transport takes place by several mechanisms including passive diffusion, carrier-mediated transport (e.g. facilitated diffusion and active transport) and vesicular transport (e.g. endocytosis, phagocytosis). On the other hand, paracellular transport in which drug diffuses through the aqueous pores at the tight junctions between the intestinal enterocytes is the minor route for absorption and controlled by the tight junctions between the epithelial cells.

Oral absorption of drugs is controlled by two main parameters namely dissolution of drug from the dosage form and the permeability of dissolved drug across the gastrointestinal membrane [7]. Rate limiting step in absorption is the permeability for compounds with low permeability, and dissolution for compounds with low solubility.

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The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. According to the BCS, drugs substances are classified into four groups: Class I with high solubility and high permeability; Class II with low solubility and high permeability; Class III with high solubility and low permeability; Class IV with low solubility and low permeability [8]. According to the FDA BCS-guidance, a drug substance is defined as highly soluble when the highest dose strength of the drug is soluble in 250 mL or less of aqueous media over the pH range of 1-6.8 at 37±1°C. In the absence of evidence suggesting instability in the gastrointestinal tract, a drug substance is considered to be highly permeable when the systemic bioavailability or extent of absorption in human (f_a) is determined to be 85% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose [9]. According to the EMA and FDA guidelines, immediate release dosage forms of BCS Class I and III compounds are subject to a BCS-based biowaiver given that all recommended criteria (For Class I: high solubility and high permeability for drug substance, rapid in vitro dissolution rate (>85% in 30 min) for test and reference products; For Class III: high solubility and limited absorption for drug substance; very rapid in vitro dissolution rate (>85% in 15 min) for test and reference drug products; no change in excipients that might affect bioavailability) are met [9,10].

According to FDA BCS-based biowaiver guidance, permeability class of a drug substance can be determined using different methods such as pharmacokinetic studies in human (mass balance or absolute bioavailability studies), and intestinal perfusion approaches (in vivo intestinal perfusion studies in humans, in vivo or in situ intestinal perfusion studies using suitable animal models, in vitro permeation studies using excised intestinal tissues, in vitro permeation studies across a monolayer of suitable cultured epithelial cells) [9]. In general, a single method is sufficient for

determination of permeability when the absolute bioavailability is $\geq 85\%$, or the administered drug excreted unchanged in urine is $\geq 85\%$, or when $\geq 85\%$ of the administered drug is recovered in urine as parent and metabolites. Before determination of the permeability class of a drug, suitability of a permeability method should be validated by using model drugs in the range of zero, low ($f_a < 50\%$), moderate ($f_a = 50 - 84\%$), and high ($f_a \geq 85\%$) absorption [9]. According to FDA guideline, sufficient number of model drugs should be used for the method validation to characterize high, moderate and low permeability, and also assay should be performed in replicates for reliable permeability results. Examples of model drugs recommended for determination of suitability of a permeability method were given in Table 1. After demonstrating suitability of a method, permeability of a drug can be determined using internal standards (a low and a high permeability model drug included in the perfusion fluid or donor fluid along with the test drug substance), and a fluid volume marker (a zero permeability compound such as PEG 4000) [9].

According to EMA guidance, reliable human investigations should be performed to justify complete drug absorption. For this purpose, data obtained from absolute bioavailability or mass-balance studies are recommended to be used to support the claim [10].

The aim of this study was to evaluate the methods in detail recommended by FDA and EMA for determination of permeability of drugs. For this purpose, different permeability methods were obtained from the guidance and literature. PubMed, Google Scholar and Web of Science search engines were used for the literature search from 1980 to 2019 using the keywords permeability determination methods, permeability from Caco-2 cells, in vivo and in situ intestinal perfusion, in vitro permeability, intestinal permeability, human intestinal perfusion, in vivo permeability, in situ permeability.

Table 1. List of model drugs recommended for determination of suitability of a permeability

Zero permeability	Low permeability ($f_a < 50\%$)	Moderate permeability ($f_a = 50 - 84\%$)	High permeability ($f_a \geq 85\%$)
Polyethylene glycol 4000	Sulpiride	Ranitidine	Minoxidil
Lucifer yellow	Nadolol	Enalapril	Disopyramide
Inulin	Enalaprilat	Terbutaline	Caffeine
Lactulose	Acyclovir	Creatinine	Phenytoin
Fluorescein isothiocyanate dextran (MW $\geq$ 3000)	Polyethylene glycol 400	Atenolol	Naproxen
	Chlorothiazide	Hydrochlorothiazide	Carbamazepine
	Lisinopril	Metformin	Propranolol
	Mannitol	Amiloride	Metoprolol
	Famotidine	Furosemide	Theophylline
	Foscarnet	Chlorpheniramine	Antipyrine
			Ketoprofen

where f_a is the extent of absorption in human

Table 2. Examples of model drugs for permeability assay method validation from Caco-2 cells [16-19].

Drug	Fa % (% fraction dose absorbed in humans)
Acetaminophen	100
Acetylsalicylic acid	100
Amiloride	58
Antipyrine	100
Aspirin	100
Atenolol	50
Caffeine	100
Carbamazepine	70
Chlorothiazide	13
Chlorpheniramine	80
Citalopram	90
Coumarin	100
Dexamethasone	100
Digoxin	81
Diltiazem HCl	99
Disopyramide	85.3
Famotidine	45
FITC-Dextran	0
Furosemide	60
Hydrochlorothiazide	70
Ibuprofen	100
Indomethacin	100
Ketoprofen	100
Labetalol	90
Lisuride	100
Mannitol	16
Metoprolol	95
Nadolol	35
Naproxen	99
Nicardipine	100
PEG 4000	0
PEG 900	10
Phenytoin	90
Pindolol	95
Propranolol	100
Ranitidine	50
Sulfasalazine	13
Theophylline	100
Verapamil	100

Results and Discussion

Mass balance studies using unlabeled, stable isotopes or a radiolabeled drug substance can be used to demonstrate the extent of absorption of a drug. The number of subjects is critical in this method for a reliable estimate of the extent of absorption [11]. The other most important parameter is the formation of metabolite. When the data obtained from mass balance studies are to be used to support complete absorption, it must be ensured that metabolites are formed after absorption. In this regard, EMA recommends that “when referring to total radioactivity excreted in urine, it should be ensured that there is no degradation or metabolism of the unchanged drug substance in the gastric or intestinal fluid. Phase 1 oxidative and Phase 2 conjugative metabolism can only occur after absorption (i.e. cannot occur in the gastric or intestinal fluid). Hence, data from mass balance studies support complete absorption if the sum of urinary recovery of parent compound and urinary and fecal recovery of Phase 1 oxidative and Phase 2 conjugative drug metabolites account for $\geq 85\%$ of the dose” [10].

Absolute bioavailability studies compare the bioavailability of the drug in systemic circulation following non-intravenous administration with the bioavailability of the same drug following intravenous administration. When the absolute bioavailability of a drug is $\geq 85\%$, additional data to document drug stability in the GI fluid is not necessary. However, such studies are expensive, requires preclinical toxicity test to confirm tolerable safety, and also potential problems due to solubility limitations [12].

The basic principle of perfusion experiments in humans (such as open and semi-open perfusion methods, well-stirred model, triple lumen tube method, multi-lumen tube method) is that the absorption is calculated from the disappearance rate of the drug from the perfused segment. In the open and semi open methods, the parallel tube model is applied as the concentration will decrease in an exponential fashion along the perfused segment. The well stirred model is the most applicable for regional perfusion techniques using the balloon approach. Also, in the triple lumen method entering perfusion solution and gastrointestinal fluids are mixed in a mixing segment, and at the distal end of the mixing segment a sample is taken which is considered to be the inlet concentration of the test segment. The absorption is then calculated from a second outlet sample taken at the end of the test segment. In the multi-lumen tube method, proximal contamination problem was avoided by using an occluding balloon proximal to the test segment. A separate tube ending aboral to the balloon provides continuous drainage and avoid proximal leakage into the test segment. All these methods are useful for generating knowledge about the direct in vivo membrane transport. However, low recovery of the nonabsorbable volume marker, and higher perfusion flow rates are the major drawbacks of these methods [13].

In vivo models (direct or indirect approach) are also developed for permeability experiments. In the direct method, the drug levels in blood or urine is determined as a function of time. When the measurement of drug concentration in blood or urine is difficult or not possible, studies can be done by indirect method. In this method, pharmacological response of drug is related to amount of the drug in body [14].

The other methods for permeability determination are the in situ

intestinal perfusion studies using suitable animal models, in vitro permeation studies using excised human or animal intestinal tissues such as Ussing-chamber technique and cell culture studies are considered appropriate for passively transported drugs [9]. These methods are more rapid and easy than the other methods, provide insights into segmental differences, and requires fewer compounds. Although Caco-2 cells are derived from human colon adenocarcinoma cells, Caco-2 cells have morphological similarity to human small intestinal enterocytes, they are polarized with an apical brush border, tight intercellular junctions, and several active transporters. Therefore, Caco-2 cells are approved by the guidelines, and commonly used in permeability determination studies [15]. Examples of model drugs for permeability assay method validation across Caco-2 cells were given in Table 2 [16-19]. Additionally, in situ models offer more realistic results than in vitro techniques due to animal's blood supply remains intact.

Conclusion

Oral bioavailability of systemically acting compounds can be limited by dissolution and permeability. For permeability limited absorption, use of the most suitable method for determination of permeability across the intestines is very important to obtain reliable results. In general, a single method may be sufficient: when the absolute bioavailability is $\geq 85\%$, or when $\geq 85\%$ of the administered drug is excreted unchanged in urine, or when $\geq 85\%$ of the administered drug is recovered in urine as parent and metabolites with evidence indicating stability in the GI tract. When a single method fails to demonstrate a permeability classification, two different methods may be used. If conflicting results are obtained from different types of studies, human data replace in vitro or animal data.

Competing interests

The authors declare that they have no competing interest.

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Ethical approval

No ethic approval is needed to this research.

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