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Review

Drug permeability profiling using cell-free permeation tools: Overview and applications

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ABSTRACT

Cell-free permeation systems are gaining interest in drug discovery and development as tools to obtain a reliable prediction of passive intestinal absorption without the disadvantages associated with cell- or tissue-based permeability profiling. Depending on the composition of the barrier, cell-free permeation systems are classified into two classes including (i) biomimetic barriers which are constructed from (phospho)lipids and (ii) non-biomimetic barriers containing dialysis membranes. This review provides an overview of the currently available cell-free permeation systems including Parallel Artificial Membrane Permeability Assay (PAMPA), Phospholipid Vesicle-based Permeation Assay (PVPA), Permeapad®, and artificial membrane based systems (*e.g.* the artificial membrane insert system (AMI-system)) in terms of their barrier composition as well as their predictive capacity in relation to well-characterized intestinal permeation systems. Given the potential loss of integrity of cell-based permeation barriers in the presence of food components or pharmaceutical excipients, the superior robustness of cell-free barriers makes them suitable for the combined dissolution/permeation evaluation of formulations. While cell-free permeation systems are mostly applied for exploring intestinal absorption, they can also be used to evaluate non-oral drug delivery by adjusting the composition of the membrane.

1. Introduction

Despite the tremendous increase in approved (bio)pharmaceutical products intended for intravenous or subcutaneous administration, the oral route of administration remains of major interest since it is beneficial from an economical, convenience and safety point of view ("2016 FDA drug approvals - nrd.2017.14.pdf," n.d.; Ecker et al., 2014). Nevertheless, before reaching the systemic circulation and, subsequently, its site of action, the orally administered drug must cross the intestinal mucosa, a major barrier for oral drug delivery.

Numerous methods exist to estimate the extent of absorption across the human gastrointestinal wall (Bohets et al., 2001; Buckley et al., 2012). Using a fairly simple computational approach, different research groups proposed to estimate drug permeation based on physicochemical drug properties including molecular weight (MW), lipophilicity (log D), acid dissociation constant (pKa), polar surface area (PSA), and hydrogen bonding potential (Camenisch et al., 1998; Neuhoff et al., 2005; Palm et al., 1997; Veber et al., 2002). These molecular descriptors, with the exception of pKa and PSA, are well covered in Lipinski's rule of 5 and provide a rational basis for understanding oral drug absorption processes in early stage drug development (Lipinski et al., 2001; Veber et al., 2002).

In contrast to this over-simplification of estimating intestinal drug permeation, tissue-based permeation models offer the advantage to closely mimic the *in vivo* situation from an anatomical, biochemical and structural point of view. For instance, the *in situ* rat intestinal perfusion technique with mesenteric blood sampling is often used for specific research scenarios aiming at (i) unravelling intestinal drug absorption mechanisms induced by drug transporters and cytochrome P450 enzymes allowing the investigation of transporter-metabolism interactions and (ii) exploring differences in regional drug absorption (Stappaerts et al., 2015; Ungell et al., 1998). Alternatively, the Ussing chambers model, in which rat or human intestinal tissue is mounted between 2 half chambers, also offers the opportunity to investigate differences in regional drug absorption, carrier-mediated transport and the impact of intestinal metabolism on drug transport (Mols et al.,

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2005; Rogers et al., 1987). Despite their high relevance for the *in vivo* situation, these tissue-based absorption systems are associated with several drawbacks including low- to moderate-throughput, limited tissue availability, ethical burdens and challenging experimental procedures. More specific drawbacks, which further impede the use of tissue-based permeation models, are the unknown effect of anesthesia on drug absorption using the *in-situ* perfusion method and the possible underestimation of drug transport due to accumulation in the irremovable circular muscle layers in the Ussing chambers model.

Cell-based systems including the human colorectal adenocarcinoma (Caco-2) cell line and the Madin Darby canine kidney (MDCK) cell line are considered as valuable alternatives to assess intestinal drug permeation. In particular, the well-established Caco-2 cell line is widely used since this system generates reproducible and biorelevant permeability results on a high-throughput basis (Balimane et al., 2000). The presumed superior predictive power of cell-based models over molecular descriptors depends on careful selection of a reference compound set (Linnankoski et al., 2008). Furthermore, this permeation model allows for the investigation of carrier-mediated transport due to the expression of intestinal uptake and efflux transporters (Matsson et al., 2015; Ölander et al., 2016). Despite their high popularity, cell-based permeation systems suffer from several shortcomings including a relative incompatibility with food components and certain pharmaceutical excipients, the absence of CYP3A4 and the lack of a mucus layer (Sun et al., 2002). Furthermore, large inter- and intra-laboratory variability in transporter expression may impair comparability of the measured permeability values (Hayeshi et al., 2008; Lee et al., 2017).

Despite the reasonable predictive power of tissue- and cell-based permeation systems for the estimation of intestinal drug permeation, these models suffer from time-consuming and expensive preparation steps. As a result, a growing interest in the development of cell-free permeation systems has evolved wherein lengthy and expensive preparation steps are drastically reduced.

The present review provides an overview of the currently available cell-free permeation systems including the parallel artificial membrane permeation assay (PAMPA), the phospholipid vesicle based permeation assay (PVPA), Permeapad® and the artificial membrane insert system (AMI-system). The main focus of this review is the description of these permeation systems in the context of oral drug delivery; however, a section on their applicability in non-oral drug delivery evaluation is also included.

2. Mechanisms of intestinal drug absorption

While drug absorption in the stomach is of minor importance, the small intestine is the main site of absorption of orally administrated drugs due to its unique anatomical properties. Particularly, the presence of (micro)villi drastically increases the surface area of the intestinal mucosa resulting in the enormous absorptive surface area of the small intestine (Helander and Fändriks, 2014; Niess and Reinecker, 2006). Drugs can cross the intestinal epithelium layer in several ways, as illustrated in Fig. 1. The type of intestinal transport is strongly connected to several physicochemical properties of the drug as described by Lipinski's rule of five which indicates whether a drug is likely to be absorbed after oral administration (Lipinski, 2000; Lipinski et al., 2001). For instance, depending on the lipophilicity of the drug, passive diffusion through the enterocytes (transcellular diffusion, Fig. 1(A)) is the preferred route for lipophilic compounds, while small hydrophilic compounds are mainly absorbed *via* passive diffusion between the enterocytes (paracellular diffusion, Fig. 1(B)) (Artursson et al., 1993; Camenisch et al., 1996). However, the contribution of passive paracellular diffusion to the overall drug transport is limited since the area available for this type of transport only accounts for 0.01% of the total surface area of the intestinal membrane (Zhu et al., 2017). In contrast to these passive routes of transport, some drugs reach the systemic circulation by means of active uptake (Fig. 1(C)), which requires energy (Tsuji and Tamai, 1996). As a result of this energy-dependence, active transport enables drug transport against a concentration gradient. Additionally, efflux transporters (Fig. 1(D)) limit intestinal drug absorption by actively transporting drugs back to the luminal environment (Chan et al., 2004; Kapitza et al., 2007). Finally, transcytosis, *i.e.* compounds migrating from the luminal to the serosal side of the intestinal epithelium layer by incorporation in vesicles from the cell membrane, may contribute to the uptake of certain drugs (Fig. 1(E)) (Florence and Hussain, 2001).

In the past decades, the majority of new chemical entities (NCEs) in the pipeline of pharmaceutical companies have increased in lipophilicity and size; as a result, many of these NCEs are preferably absorbed by passive transcellular diffusion (Fig. 1(A)). Presently, 80%–95% of the commercially available drugs are mainly absorbed transcellularly (Mandagere et al., 2002), justifying the development of time-and cost-effective cell-free permeation systems. It should be noted, however, that these cell-free permeation tools could be exclusively applied for predicting passive transcellular drug transport; paracellular and active drug transport cannot be captured.

3. Currently available cell-free permeation systems and their predictive capacity

Depending on the composition of the barrier, cell-free permeation systems are typically classified into two classes including (i) biomimetic barriers which are constructed from (phospho)lipids and (ii) non-biomimetic barriers containing dialysis membranes. Below, an overview of the currently available cell-free permeation systems is provided in terms of their barrier composition. In addition, their predictive capacity is discussed in relation to well-characterized permeation systems.

3.1. Parallel artificial membrane permeation assay (PAMPA)

3.1.1. Original PAMPA

In 1998, PAMPA was introduced for the first time when the Roche team presented the use of artificial membranes in a 96 well microtiter plate format, as illustrated in Fig. 2 (Kansy et al., 1998). PAMPA barriers generally consist of a filter (*e.g.* polyvinylidene fluoride (PVDF)) soaked with (phospho)lipids dissolved in an organic solvent. Since the PAMPA barrier does not contain a physical boundary separating the donor media from the lipophilic barrier constituents, potential dissolution/emulsification of barrier constituents into the media may occur. Initially, an n-dodecane solution of egg lecithin (1–20%) (a mixture of lipids containing phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and cholesterol) was used to mimic the phospholipid composition of the mammalian membrane. Using this composition, Kansy and co-workers were able to correlate measured PAMPA fluxes at different pH values (6.5 and 7.4) with the fraction absorbed in humans (Kansy et al., 1998). Although the relationship obtained between the measured permeability values and the fractions absorbed in humans was similar to what was described for Caco-2 permeation studies, PAMPA suffers from the inability to predict paracellular and active transport, and potential membrane retention of lipophilic compounds. However, the contribution of paracellular transport to overall drug transport can be addressed using additional *in silico* models, which simulate the characteristics of the human epithelium; this approach may avoid underestimation of the fraction absorbed of small, hydrophilic molecules like atenolol, metformin, cimetidine or terbutaline (Adson et al., 1994; Sugano et al., 2002).

3.1.2. Variants of PAMPA

In the following years, several variations of the setup were published, which reflect tissues in the human body containing different lipid compositions (Proulx, 1996). The variants of the original PAMPA assay, which are listed in Table 1, differ by the nature of the filter support, the composition of the membrane constituents, the pH in