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Biomimetic PVPA *in vitro* model for estimation of the intestinal drug permeability using fasted and fed state simulated intestinal fluids

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ABSTRACT

A prerequisite for successful oral drug therapy is the drug's ability to cross the gastrointestinal barrier. Considering the increasing number of new chemical entities in modern drug discovery, reliable and fast *in vitro* models are required for early and efficient prediction of intestinal permeability. To mimic the intestinal environment, use of biorelevant media may provide valuable information on *in vivo* drug permeation. The present study aims at improving the novel biomimetic phospholipid vesicle-based permeation assay's ($PVPA_{biomimetic}$) biorelevance by investigating the applicability of the biorelevant media; fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF). The FaSSIF and FeSSIF's influence on the permeability of the model drugs acyclovir, indomethacin, griseofulvin and nadolol was then assessed. The barriers' robustness in terms of storage stability was also evaluated. The barriers were found to maintain their integrity in presence of FaSSIF and FeSSIF. The model drugs showed changes in permeability in presence of the different simulated intestinal fluids that were in agreement with previous reports. Moreover, the barrier showed improved storage stability by maintaining its integrity for 6 months. Altogether, this study moves the $PVPA_{biomimetic}$ an important step towards a better *in vitro* permeability model for use in drug development.

1. Introduction

Although several routes of administration are available to bring a drug to the desired site of action, oral administration is still the most important and prevalent route of administration, due to its cost efficiency, convenience and patient compliance (Kanzer et al., 2010). Modern drug discovery programs are often based on combinatorial chemistry and high-throughput screening, and a majority of the new drug candidates are suffering from poor water solubility (Augustijns et al., 2014). It is recognized that up to 90% of the new chemical entities are classified as Class II and IV in the Biopharmaceutics Classification System (BCS) (Sjogren et al., 2013). Drug compounds that are classified as Class II are suffering from poor solubility, but are highly permeable, while the class IV compounds suffer both from insufficient solubility and permeability (Dressman and Reppas, 2000). Insufficient oral bioavailability of a drug is usually associated with limited solubility and/or permeability (Flaten et al., 2011).

Consequently, appropriate and reliable high throughput *in vitro* models to assess absorption and distribution potential of new drug candidates and drug formulations early in the development process are a prerequisite for increased success rate as well as reduced time and costs (Lennernas, 2014). Recently, approval of a new European project within the Innovative Medicines Initiative (IMI) program in the area of oral biopharmaceutics tools (OrBiTo) underlines the importance of this issue. The project aims at improving the understanding of how orally administered drugs are absorbed from the gastro intestinal tract (GI) and simultaneously apply this information to develop new advanced tools applicable in permeability screening (Sjogren et al., 2014).

A number of *in vitro* models for the prediction of intestinal absorption, able to provide information on these aspects early in

Abbreviations: BCS, biopharmaceutics classification system; Chol, cholesterol; CMC, critical micelle concentration; E-80, egg phospholipids; ER, electrical resistance; FaB, fasted state buffer; FaSSIF, fasted state simulated intestinal fluid; FeB, fed state buffer; FeSSIF, fed state simulated intestinal fluid; GI, gastrointestinal tract; HPLC, high performance liquid chromatography; OrBiTo, oral biopharmaceutics tools; PAMPA, parallel artificial membrane permeability assay; PB, phosphate buffer; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PVPA, phospholipid vesicle-based permeation assay; $PVPA_{biomimetic}$, PVPA with PC, PE, PS, PI and Chol in the barrier; $PVPA_{o}$, PVPA with E-80 in the barrier.

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the development process, have been established. Currently, the common standard in *in vitro* permeability screening which aims at simulating the intestinal epithelia is the cell-based Caco-2 model, which has been extensively used in both academia and industry (Artursson, 1990; Artursson et al., 2001; Bergstrom et al., 2009). This model is of special value in estimating the impact of active transport proteins on the permeability of orally administered drugs. However, cell-based models might be too complicated, time consuming, and costly in regard to modern high-throughput requirements. In addition, the reproducibility and stability of this cell-based model might be prone to variations as the culturing cells are living systems (Gantzsch et al., 2014; Sambuy et al., 2005). Another currently established model designed for rapid screening of drug permeability is the parallel artificial membrane permeation assay (PAMPA) (Avdeef, 2003a; Kansy et al., 1998; Wohnsland and Faller, 2001). PAMPA generally consists of a filter support covered with phospholipids in organic solvents, which allows for medium- to high-throughput screening of drug permeability. However, lack of resemblance with the biological membrane structure, few reports on use in permeability screening from different formulations and limited knowledge on use of more biorelevant media is placing a limitation on this model (Flaten et al., 2006b; Markopoulos et al., 2014).

Recently, a new addition to the phospholipid vesicle-based permeation assay (PVPA) family, the PVPA with a biomimetic lipid composition ($PVPA_{biomimetic}$), has been introduced by us for better assessment of passive intestinal permeability (Naderkhani et al., 2014). The PVPA developed for the screening of passive permeability, consists of a tight barrier of liposomes on a filter support. The $PVPA_{biomimetic}$ established to be an intestinal mimicking barrier, has proven to be tremendously more robust towards the presence of tensides and co-solvents compared to the original PVPA ($PVPA_o$), granting the model an enhanced ability to estimate the permeability of poorly soluble compounds. This is of great importance since many drug candidates today are suffering from poor water solubility (BCS class II or IV drugs) (Bergstrom et al., 2014).

Another important point in permeability estimation is to utilize more biorelevant media to mimic the *in vivo* conditions in the test set up. It is known that drugs from different classes might be affected differently in the presence of biorelevant fluids and also the presence of certain components in the GI fluid such as lecithin and bile salt (sodium taurocholate) might have a large impact on the solubility and permeability of poorly soluble compounds (Bergstrom et al., 2014; Dahan and Miller, 2012). Among the biorelevant media, FaSSIF and FeSSIF, first introduced in 1998 by Dressman and co-workers and later modified to better predict *in vivo* behavior of drugs, are the most utilized fluids originally applied as dissolution medium and later employed as medium in the permeability screening (Galia et al., 1998; Jantratid et al., 2008). FaSSIF has previously been shown to be compatible with the original $PVPA_o$-barrier consisting of egg phospholipids (Fischer et al., 2012) However, the more challenging FeSSIF has never been tested in any of the PVPA models.

The aim of this study was thus to improve the $PVPA_{biomimetics}$ biorelevance by i) investigating the applicability of the biorelevant media FaSSIF and FeSSIF in the novel $PVPA_{biomimetic}$ model and ii) evaluating FaSSIF and FeSSIF's influence on the permeability of model drugs (acyclovir, indomethacin, griseofulvin, nadolol) with a diverse set of physicochemical properties leading to classification into different BCS classes. In addition, the $PVPA_{biomimetic}$ barrier robustness in terms of the storage stability was evaluated to improve the assay's user-friendliness.

2. Materials and methods

Acetonitrile CHROMASOLV®, acycloguanosine, cholesterol (Chol), Fiske-SubbaRow reducer, phosphatidylinositol (PI), formic acid eluent additive for LC-MS, griseofulvin, glyceryl monooleate, indomethacin, maleic acid, methanol CHROMASOLV®, nadolol, potassium phosphate monobasic, sodium chloride, sodium dihydrogen phosphate, sodium hydroxide, sodium oleate and sodium taurocholate were obtained from Sigma–Aldrich, Steinheim, Germany. Ammonium molybdate, hydrogen peroxide, and disodium hydrogenphosphate dihydrate were purchased from Merck KGaA, Darmstadt, Germany. Lipoid egg phospholipids (E-80), egg phophatidylcholine (PC), egg phosphatidylethanolamine (PE), egg phosphatidylserine (PS) were obtained from Lipoid, Ludwigshafen, Germany. Sulphuric acid was provided by May and Baker LTD, Dagenham, England. All chemicals used in the experiments were of analytical grade.

Filter inserts (Transwell®, $d = 6.5$ mm) and plates were purchased from Corning Inc., Corning, New York, USA. The mixed cellulose ester filters (0.65 μm pore size) were purchased from Millipore, Billerica, Massachusetts, USA. Whatman® nucleopore track-etch membrane filters (0.4 μm, 0.8 μm and 1.2 μm pore size) were obtained from Whatman, part of GE Healthcare, Little Chalfont, UK.

2.1. Preparation of liposomes for the $PVPA_{biomimetic}$ barriers

Liposomes were prepared using the film hydration method as previously described (Naderkhani et al., 2014). Briefly, two different lipid compositions were used:

1. E-80 (100%) for liposomes to fill the pores of the filter in the first part of the preparation procedure.
2. PC (26.5%, w/w), PE (26.5%, w/w), PS (7%, w/w), PI (7%, w/w), Chol (33%, w/w), for liposomes for the layer on top of the filter in the last part of the preparation procedure.

The lipids were dissolved in a mixture of chloroform and methanol (2:1, v/v) in a round bottom flask. The organic solvents were removed by evaporation. The dried lipid films were then hydrated with phosphate buffer (PB pH 7.4), followed by the addition of 10% (v/v) ethanol to achieve a 6% or 3% (w/v) liposomal dispersion for composition 1 and 2, respectively (Flaten et al., 2006b; Naderkhani et al., 2014). The liposomes were then extruded by hand using either syringe filter holders or Lipofast (Avastin Europe GmbH, Mannheim, Germany) to obtain liposomes of different size distributions as previously described (Naderkhani et al., 2014).

2.2. Preparation of the $PVPA_{biomimetic}$ barriers

The $PVPA_{biomimetic}$ barriers, designed to study the permeability from the intestinal epithelia, were prepared according to the procedure previously described (Naderkhani et al., 2014). In brief, the procedure was as follows:

- Addition of small liposomes (extruded through 400 nm pore filter) of E-80 to fill the pores of the filter support: liposomes (100 μl) were deposited on the filter support (650 nm pores) and centrifuged at 610*g* for 5 min. The procedure was repeated with addition of liposomes (100 μl) and centrifuged for 10 min.
- Heating of barriers at 50 °C for 45 min.