Development of a Biomimetic Phospholipid Vesicle-based Permeation Assay for the Estimation of Intestinal Drug Permeability

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ABSTRACT: Permeability is a crucial property of orally administered drugs. Therefore, in drug discovery, it is important to employ methods suitable for rapidly and reliably screening the permeability of large numbers of new drug candidates. The phospholipid vesicle-based permeation assay (PVPA), a model consisting of a tight layer of liposomes immobilized on a filter, offers potential advantages unmet by other methods and has been successfully used in permeability testing of novel active substances as well as formulations. In this study, the PVPA was developed into a more robust, biomimetic model by employing a lipid composition matching that of the intestinal permeation barrier and performing the experiments at the more biologically relevant pH 6.2. As expected, positively charged basic compounds demonstrated increased permeability through the negatively charged biomimetic barriers, and the degree of correct classification according to *in vivo* absorption was comparable between the original PVPA and the biomimetic PVPA. The biomimetic PVPA further proved to be tremendously more robust toward the presence of tensides compared with the original PVPA; this is a promising finding that renders the biomimetic PVPA an enhanced ability to estimate the permeability of poorly soluble compounds. Hence, the PVPA model developed in this study has evolved an important step forward. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1882–1890, 2014

Keywords: *in vitro* model; liposome; lipid; artificial membrane; intestinal absorption; screening; tenside; cosolvent; absorption; surfactants

INTRODUCTION

Assessment of the oral bioavailability of new drug candidates in the early stages of drug development is of special interest as modern drug discovery regimens produce hundreds of thousands of potential drug candidates for high-throughput screening for activity or potency related characteristics. There is thus a rapidly increasing demand for *in vitro* models that can reliably assess drug absorption and disposition, preferably in a high-throughput test format.

Both *in silico* and experimental methods are currently available for the prediction and/or screening of properties related to oral drug absorption. The gold standard in *in vitro* permeability screening is currently the cell-based Caco-2 model, which is widely established in both academia and industry.[1–3] 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, such as the Caco-2 model or the novel method of coculturing three different cell lines to obtain a more complete picture of oral absorption,[4] might be too complicated and time consuming with regard to modern high-throughput requirements. One of the most commonly used current techniques for the rapid screening of drug permeability through the intestinal epithelia is the parallel artificial membrane permeability assay (PAMPA).[5–10] PAMPA uses a simple phospholipid/organic solvent-coated filter as the permeability barrier, which allows for medium- to high-throughput screening of permeability properties. Such simplified permeability approaches model only the passive pathway, not paracellular or active transport pathways. However, reports conclude that 80%–95% of the commercial drugs are absorbed primarily by passive diffusion.[11] Therefore, a robust model focused on passive diffusion, not relying on cells and thus more compatible with high-throughput applications appears to be a useful first step in the biopharmaceutical characterization of new chemical entities.

We originally reported the phospholipid vesicle-based permeation assay (PVPA), a predictive medium- to high-throughput screening method for passive drug permeability based on a tight barrier of liposomes on a filter support.[12] To the best of our knowledge, this was the first successful attempt to deposit a lipid-based membrane barrier without the use of an inert solvent, such as hexadecane. The PVPA barriers were originally prepared from egg phosphatidylcholine liposomes placed onto a filter support by centrifugation. Solvent evaporation and freeze–thaw (ft) cycling were then used to promote liposome fusion, resulting in a tight barrier consisting of layers of liposomes mimicking a cell layer/tissue. The PVPA was validated using a selection of compounds and the apparent permeability coefficients obtained correlated well with literature data on human absorption *in vivo*, which suggested that the method is suitable for rapidly screening of the passive transport of drugs and new chemical entities.[12] The PVPA approach appears to, under

Abbreviations used: Chol, cholesterol; E-80, egg phospholipids; PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol; PS, phosphatidylserine; PAMPA, parallel artificial membrane permeability assay; ft, freeze–thaw; PBS, phosphate-buffered saline; PVPA, phospholipid vesicle-based permeation assay: $PVPA_0$ consisting of E-80, $PVPA_{biomimetic}$, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserin, phosphatidylinositol, cholesterol.

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comparable settings, mimic *in vivo* absorption better than the biomimetic PAMPA model[8] and performs equally well in comparison with the Caco-2 cell model[13] and the double sink PAMPA (DS-PAMPA) model[5] in predicting the passive diffusion of drug compounds.[12] The model has further been shown to be adaptable to automation using a laboratory robotic system[14] and is stable at −80°C for up to 2 weeks,[15] drastically increasing throughput. The functionality of the barriers has also been demonstrated to be stable within a pH range of 2.0–8.0, which makes the barriers suitable for further studies to provide, for example, insight into segmental absorption in the human gastrointestinal tract or to mimic other absorption barriers with a pH environment different from pH 7.4.[15] The PVPA has further been used to test the permeability of new active substances as well as drugs in formulations. The oral permeability of antibacterial peptides has been predicted[16–18] and different formulations, that is, micelles, solid solutions, and liposomes, have been tested in our model.[19,20] Recently, a modified PVPA mimicking healthy and compromised skin barriers has been introduced.[21]

The goal of an *in vitro* permeability model is the ability to predict permeability values that correlate well with *in vivo* permeability data. To accomplish this, it is important that the model mimics the *in vivo* situation as closely as possible. Factors receiving much attention in this respect include the pH profile of the gastrointestinal tract and the lipid composition of the artificial barriers. It has been shown that membranes consisting of only phosphatidylcholine (PC) underestimate the permeability of basic and acidic drugs and a better *in vivo–in vitro* correlation can be obtained using a negatively charged barrier with a biomimetic lipid composition.[8,22]

Furthermore, an increasing problem in drug development is that a large number of new drug candidates demonstrate low water solubility, which may result in problems pertaining to permeability and bioavailability. To overcome this challenge, solubility-increasing agents can be added to the donor phase to increase drug solubility. It is therefore also important that a permeability model maintains its integrity in the presence of the solubility-enhancing agents of interest.

The aim of this study was thus to improve the biomimetic properties of the PVPA model by first investigating whether the use of more biologically relevant pH conditions in the permeability experiments would lead to improved prediction of *in vivo* absorption. Second, a biomimetic lipid composition was designed to better mimic the intestinal barrier in an effort to significantly influence the permeation of drugs. A change in the lipid composition could potentially provide a more robust barrier capable of handling harsher conditions, which in turn would enable an estimation of the permeability of poorly soluble compounds. Finally, the stability of the biomimetic PVPA in the presence of tensides and cosolvents was investigated.

MATERIALS AND METHODS

Materials

Egg phosphatidylcholine, lipoid egg phospholipids (E-80), egg phosphatidylethanolamine (PE), and egg phosphatidylserine (PS) were obtained from Lipoid, Ludwigshafen, Germany. Cholesterol (Chol), phosphatidylinositol (PI), methanol, alprenolol hydrochloride, atenolol, acebutolol hydrochloride, caffeine, chloramphenichol, chlorothiazide, enalapril maleate, hydrochlorothiazide, metoprolol tartrate, nadolol, ranitidine hydrochloride, sulfasalazine, sulpiride, testosterone, terbutaline hemisulfate, timolol maleate, tranexamic acid, calcein, ibuprofen, flufenamic acid, flubiprofen, and metronidazole were purchased from Sigma–Aldrich Company, St. Louis, Missouri. Amiloride hydrochloride and chloroform were supplied by Merck KGaA, Darmstadt, Germany. Salicylic acid and acetyl salicylic acid were obtained from NMD, Oslo, Norway. Cimetidine was purchased from Allphamed Pharma GmbH, Göttingen, Germany.

Propranolol hydrochloride was purchased from TCI Europe N.V., Zwijndrecht, Belgium. Diclofenac sodium was purchased from AWD Pharma GmbH & Company, KG, Dresen, Germany.

Filter inserts (Transwell, $d = 6.5$ mm) and plates were purchased from Corning Inc., Corning, New York, and the mixed cellulose ester filters (0.65 μm pore size) were obtained from Millipore, Billerica, Massachusetts.

Liposome Preparation

Liposomes were prepared using the film hydration method. Lipids were dissolved in a mixture of chloroform and methanol (2:1) in a round bottom flask. The organic solvent was removed under vacuum at 45°C. The deposited lipid film was exposed to a vacuum of 55 hPa at room temperature for an additional 3 h period to remove all traces of solvent prior to hydration with phosphate buffer containing 10% (v/v) ethanol, thereby yielding a 6% (w/v) liposomal dispersion as previously described.[12] The liposomes were then extruded by hand using either syringe filter holders or Lipofast (Avestin Europe GmbH, Mannheim, Germany).

Liposome size distributions were measured using photon correlation spectroscopy in a Submicron Particle Sizer 370 (PSS Nicomp Particle Sizing Systems, Santa Barbara, California). Sample preparation and measuring conditions were as previously described.[23] Three cycles of 15-min measurements were performed.

Zeta potential measurements were performed on liposome dispersions diluted in phosphate-buffered saline (PBS) pH 7.4 using a Malvern Zetasizer nano Z (Malvern, Worcestershire, UK).

The Original Preparation Method for the PVPA Barriers

The PVPA barriers were prepared according to the procedure previously described[12] and were stored for up to two weeks at −80°C, in accordance with previous stability studies.[15] In brief, liposome dispersions with a mean size of 300 nm (volume weight) and 600 nm (number weight) were deposited on a filter support by centrifugation. The liposomes were added in consecutive steps from smallest to largest. ft cycling was then used to promote fusion of the liposomes and hence produce a tight barrier.[12]

Permeation Studies

Permeation studies were performed using solutions of drugs or hydrophilic markers in phosphate buffer (pH 7.4 or 6.2), according to the procedure previously described.[12] Briefly, inserts were loaded with drug solution (100 μL) and placed in separate acceptor compartments containing phosphate buffer (600 μL). During the first 3 h of the study, the loaded inserts were moved to wells containing equal quantities of fresh buffer at 1 h intervals; over the subsequent 2 h, the inserts were moved at 0.5 h intervals. For cimetidine, nadolol, chlorothiazide, and hydrochlorothiazide, the inserts were moved every second hour