European Journal of Pharmaceutics and Biopharmaceutics 88 (2014) 840–846

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

A new *in vitro* system for evaluation of passive intestinal drug absorption: Establishment of a double artificial membrane permeation assay

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ARTICLE INFO

Article history:
Received 16 January 2014
Accepted in revised form 28 September 2014
Available online 7 October 2014

Keywords:
Artificial membrane
Permeability
Oral absorption
Human intestinal permeability
Renkin function
In vitro assay

ABSTRACT

The aim of this present study was to establish a new *in vitro* assay, double artificial membrane permeation assay (DAMPA), to evaluate the human intestinal permeability of drugs. A double artificial membrane with an intracellular compartment was constructed in side-by-side chambers by sandwiching a filter containing buffer solution with impregnated lipophilic filters with dodecane containing 2 w/v% phosphatidylcholine. Permeation data of ionic compounds clearly indicated that not only the pH value of the apical solution but also that of the intracellular compartment affected the permeability across the double artificial membrane. DAMPA was performed with 20 compounds at physiological pH (apical; 6.5, intracellular and basal; 7.4). Paracellular and transcellular permeabilities of compounds in human epithelium were estimated based on the characteristics of the paracellular pathway using physicochemical properties of compounds with the Renkin function and the area factor i.e. the difference in the effective surface area between human epithelium and the double artificial membrane, respectively. The human intestinal permeability of each compound was predicted by the sum of estimated transcellular and paracellular permeabilities. Predicted human intestinal permeability was significantly correlated with the fraction of absorbed dose in humans, indicating that DAMPA has the potential to predict oral absorption of drugs in humans.

1. Introduction

Partitioning of drugs into organs, tissues or cells and drug permeability across cell membranes dominate the distribution of administered drugs into the body. Focusing on oral drug absorption, the intestinal permeability of drugs is one of the key factors in determining the fraction of absorbed dose. So far, various *in vitro* and *in situ* systems to evaluate intestinal drug permeability have been reported. Monolayers of Caco-2 cells originating from human colorectal adenocarcinoma [4,3,14], and Mardin-Darby canine kidney cells [5] are widely used as potent *in vitro* model to predict oral absorption in humans.

In the last decade and a half, many researchers have reported using an artificial membrane to evaluate intestinal permeability of compounds. The parallel artificial membrane permeation assay (PAMPA) was first reported by Kansy et al. [6]. PAMPA can be done by measuring the permeation of compounds across a hydrophobic filter soaked with phospholipids in an organic solvent. As phospholipids and organic solvents, phosphatidylcholine and dodecane were initially used [6]. Sugano et al. [10] modified the organic solvent from dodecane to 1,7-octadiene and the lipid composition from phosphatidylcholine to a mixture of different phospholipids and cholesterol to mimic the intestinal membrane, which had a significant effect on the permeability of charged compounds. The permeability of ionic compounds in not only cell-based membrane such as Caco-2 cells but also the artificial membrane is significantly affected by the pH of solutions, which led to pH-partition theory. The physiological pH of the apical and basal solution should thus be reflected in PAMPA [15,11].

When permeating an artificial membrane, ionic compounds undergo only one process of permeation across a lipid layer containing organic solvents. However, when compounds permeate

Abbreviations: DAMPA, double artificial membrane permeation assay; DMSO, dimethylsulfoxide; D/P system, dissolution/permeation system; PAMPA, parallel artificial membrane permeation assay; PC, egg-phosphatidylcholine.

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http://dx.doi.org/10.1016/j.ejpb.2014.09.009
0939-6411/

across a cell-based membrane via the transcellular route, there are two permeation processes across the lipid bilayer membrane, i.e., from the apical side to the intracellular compartment and from the intracellular compartment to the basal side. It could be considered that the intracellular pH (physiological pH, 7.4) affects the permeation of ionic compounds from the intracellular compartment to the basal side. Therefore, in order to predict the intestinal permeability of drugs using artificial membranes, the permeation of compounds across two lipid membranes reflecting the physiological pH conditions of the apical and basal sides and the intracellular compartment should be evaluated.

In this study, a double artificial membrane with an intracellular compartment, and a permeation assay using this membrane called the double artificial membrane permeation assay (DAMPA), were established. In addition, the human intestinal permeabilities of 20 compounds were predicted using the *in vitro* permeability obtained from DAMPA and the physicochemical properties of each compound.

2. Material and methods

2.1. Materials

Membrane filters (Durapore®) were obtained from Millipore Corporation (Bedford, MA). Dodecan, egg-phosphatidylcholine (PC), and dimethylsulfoxide (DMSO) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acyclovir, alprenolol, atenolol, cimetidine, metoprolol, nadolol, pindolol, piroxicam, terbutaline and warfarin were obtained from Sigma-Aldrich (St. Louis, MO). Antipyrine, carbamazepine, ketoprofen, propranolol, salicylic acid and sulpiride were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Chlorothiazide, famotidine, methotrexate and sulfasalazine were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

2.2. Permeation study

A dissolution/permeation system (D/P system), consisting of the apical and basal chambers, was used for the permeation study. The volumes of the apical and basal sides were set to 8 mL and 5.5 mL, respectively. According to the following procedure, a double artificial membrane was prepared in the D/P system (Fig. 1). Filter paper containing 50 mM sodium phosphate buffer with 5% DMSO (pH 6.5 or 7.4) sandwiched between the filters impregnated with lipid solution composed of PC (2%) and dodecane (98%) and mounted between the chambers. The apical and basal sides of the double artificial membrane were filled with 50 mM sodium phosphate buffer (pH 6.5 or 7.4) containing 5% DMSO with 100 μM of each compound and 50 mM of sodium phosphate buffer (pH 7.4) containing 5% DMSO, respectively. Both solutions were stirred by a magnetic stirring system (Scinics Corp., Tokyo, Japan) at 200 rpm. Aliquots of samples were then taken from the basal solution at appropriate intervals over 2 h. The volume of the basal solution was maintained by adding fresh basal solution. All experiments were performed at 37 °C.

2.3. Analytical methods

The concentrations of all compounds in the sample were determined using a UPLC system (ACQUITY® UPLC, Waters, MA) equipped with a tandem mass spectrometer (ACQUITY® TQD, Waters, MA). A reverse-phase Waters Acquity® UPLC BEH C18 analytical column of 50 mm length × 2.1 mm I.D. and 1.7 μm particle size (Waters, MA) was used with a mobile phase consisting of 0.1% (*v*/*v*) formic acid in water (solvent A) and acetonitrile containing 0.1% (*v*/*v*) formic acid (solvent B) for positive mode or a mobile phase consisting of 5 mM ammonium acetate in water (solvent A) and acetonitrile (solvent B) for positive or negative mode with a gradient time period. The initial mobile phase was 98% solvent A and 2% solvent B pumped at a flow rate of 0.3 mL/min. Between 0 and 1.0 min, the percentage of solvent B was increased linearly to 95%, where it was held for 1.0 min. Between 2.01 and 2.5 min, the percentage of solvent B was decreased linearly to 2%. This condition was maintained until 3 min, at which time the next sample was injected into the UPLC system. All treated samples were injected as 5 μL into the UPLC system. The ion detection conditions used to determine the concentration of each compound are listed in Table 1.

2.4. Data analysis

2.4.1. Calculation of permeability of various compounds in DAMPA

The permeability (apparent permeability, $P_{\text{trans DAMPA}}$ (cm/s)) of each compound was calculated according to the following equation:

$$P_{\text{app}} = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_0}$$

where dQ/dt is the appearance rate of compounds in the basal solution (μmol/s), C_0 is the initial concentration of compound in the apical side (μM) and A is the surface area of the membranes (cm^2). The initial concentration of all compounds and surface area were 100 μM and 1.77 cm^2, respectively.

2.4.2. Prediction of human intestinal permeability

Prediction of human intestinal permeability from DAMPA data was performed in a manner similar to that established previously by Kataoka et al. [9].

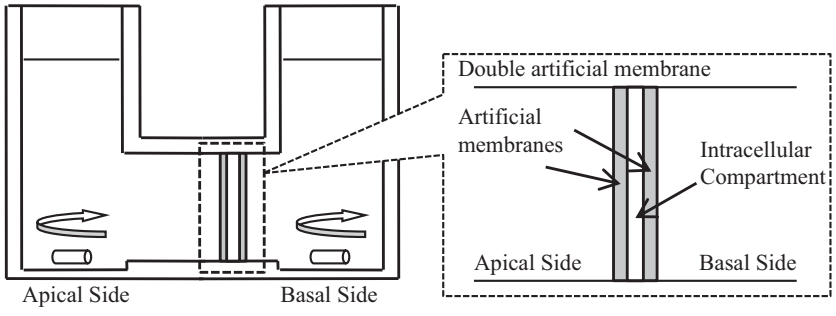

Fig. 1. Scheme of double artificial membrane permeation assay (DAMPA) constructed with the dissolution/permeation system.