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Research paper

Assessment of absorption potential of poorly water-soluble drugs by using the dissolution/permeation system

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ABSTRACT

This study aims to assess the absorption potential of oral absorption of poorly water-soluble drugs by using the dissolution/permeation system (D/P system). The D/P system can be used to perform analysis of drug permeation under dissolution process and can predict the fraction of absorbed dose in humans. When celecoxib at 1/100 of a clinical dose was applied to the D/P system, percentage of dose dissolved and permeated significantly decreased with an increase in the applied amount, resulting in the oral absorption being predicted to be 22–55%. Whereas similar dissolution and permeation profiles of montelukast sodium were observed, estimated absorption (69–85%) was slightly affected. Zafirlukast absorption (33–36%) was not significantly affected by the dose, although zafirlukast did not show complete dissolution. The relationship between clinical dose and predicted oral absorption of drugs corresponded well to clinical observations. The limiting step of the oral absorption of celecoxib and montelukast sodium was solubility, while that of zafirlukast was dissolution rate. However, due to high permeability of montelukast, oral absorption was not affected by dose. Therefore, the D/P system is a useful tool to assess the absorption potential of poorly water-soluble drugs for oral use.

1. Introduction

Recently, the number of active pharmaceutical ingredients (APIs) with poor water solubility has increased [1]. Their limited solubility often causes poor and variable oral absorption because the dissolution rate or solubility is insufficient for APIs to dissolve completely in the gastrointestinal (GI) tract. Therefore, dissolution of APIs and drugs with poor water solubility and high permeability, which are classified into class 2 by the Biopharmaceutics Classification System (BCS), in GI fluid may have a great impact on their oral absorption. Many researchers have revealed that the oral absorption of BCS class 2 drugs can be further categorized into two groups: solubility-limited and dissolution rate-limited [2–4]. The rate-limiting steps of oral absorption of such drugs can be determined by the physicochemical properties, such as solubility, dissolution rate and permeability, and the dose.

As clinical study for the development of oral dosage forms, a dose escalation trial is performed in a phase 1 study with a few male healthy volunteers. In this phase, non-linear pharmacokinetics (PK) with an increase in the dose may be observed. This non-linear PK can be classified into two types, which are that the dose-normalized systemic exposure increases or decreases with increasing dose. As a reason for the former case, because metabolic enzymes and/or efflux transporters mainly regulate the PK of drugs, the systemic exposure increases due to saturation of the metabolism and/or excretion by efflux transporters [5]. As a reason for the latter case, it is considered that the fraction of absorbed dose (Fa) decreases with increasing dose owing to poor solubility [6]. Therefore, the Fa of APIs at each dose and the maximum absorbable dose (MAD) should be assessed before clinical study in order to achieve the effective development of candidates for oral use.

We have developed an *in vitro* system for the evaluation of drug permeation under dissolution process in oral drug absorption [7]. This system (dissolution/permeation system, D/P system) consists of apical and basal chambers with a Caco-2 cell monolayer mounted between the two chambers as a model membrane of the human intestine. It was demonstrated that the D/P system using biorelevant fluids has high predictability for the oral absorption of poorly water-soluble drugs since dissolution and permeation, which occur sequentially in the GI tract, can be measured under physiological conditions similar to those in the human GI tract [8–10].

In this study, in order to elucidate further advantages of the D/P system for prediction of the oral absorption of poorly water-soluble drugs, rate-limiting steps of oral absorption in humans were assessed using the oral absorption estimated from the D/P system;

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then, the results were compared to *in vivo* observation in humans. In addition, the relationship between rate-limiting steps of oral absorption of poorly water-soluble drugs and MAD was discussed.

2. Experimental

2.1. Materials

A human colorectal adenocarcinoma cell line, Caco-2 cells (passage: 17 times), was purchased from American Type Culture Collection (Rockville, MD). Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Sigma–Aldrich (St. Louis, MO). Nonessential amino acids (10 mM), fetal bovine serum (FBS), L-glutamine (200 mM), trypsin–EDTA (trypsin: 0.25%, EDTA: 1 mM), and antibiotic–antimycotic (penicillin: 10,000 U/mL, streptomycin: 10 mg/mL, amphotericin B: 25 μg/mL; dissolved in 0.85% (w/v) sodium chloride aqueous solution) were purchased from Gibco Laboratories (Lenexa, KS). Cell culture inserts with polyethylene terephthalate filters (pore size: 3.0 μm, growth area: 4.20 cm^2) were obtained from Becton Dickinson Bioscience (Bedford, MA).

Bovine serum albumin (BSA), celecoxib, egg-phosphatidylcholine (lecithin), sodium taurocholate, and zafirlukast were obtained from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Montelukast sodium was purchased from Sigma–Aldrich (St. Louis, MO). ACCOLATE® tablets at 40 mg (zafirlukast), Celecox® tablets at 100 mg (celecoxib), and SINGULAIR® tablets at 10 mg (montelukast sodium) were obtained from Astra Zeneca Japan (Osaka, Japan), Astellas Pharma Inc. (Tokyo, Japan) and Merck & Co., Inc. (Tokyo, Japan), respectively. WellSolve was purchased from Celeste Corp. (Tokyo, Japan).

2.2. In vitro experiments with the D/P system

2.2.1. Preparation of Caco-2 cell monolayer

Caco-2 cells were cultured in DMEM supplemented with 10% (v/v) FBS, 1% (v/v) nonessential amino acids, 1% (v/v) L-glutamine, and 0.5% (v/v) antibiotic–antimycotic mixture (BCM) in a flask of adequate volume (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) at 37 °C in a humidified air with a 5% CO_2 atmosphere. Caco-2 cells were harvested using trypsin–EDTA. Collected cells were seeded on the cell culture inserts at a density of 3×10^5 cells/insert. Fresh BCM (1.5 mL in the insert and 2.6 mL in the well) was replenished every 48 h during the initial 6 days and every 24 h after the period. After 18–21 days in culture, the Caco-2 monolayer was utilized for the following experiments.

2.2.2. Chambers for the D/P system

In the D/P system, the Caco-2 cell monolayer is mounted in side-by-side chambers. The effective surface area of the Caco-2 cell monolayer in the D/P system is 1.77 cm^2. Both sides of the Caco-2 cell monolayer are consistently stirred at 200 rpm with magnetic stirrers. The volumes of apical and basal sides are set to 8 mL and 5.5 mL, respectively. As a buffer solution in this study, Hank's balanced salt solution (HBSS), containing 5.36 mM KCl, 136.89 mM NaCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.17 mM $NaHCO_3$, 1.26 mM $CaCl_2$, 0.49 mM $MgCl_2$, 0.41 mM $MgSO_4$, and 25 mM glucose, was used (transport medium, TM). Simulated intestinal medium as the apical side of the D/P system was prepared, which was based on TM with the addition of sodium taurocholate (3 mM) and lecithin (0.75 mM) for $FaSSIF_{mod}$. The pH of $FaSSIF_{mod}$ was adjusted to 6.5 with HEPES. As a basal medium, TM containing BSA (4.5% w/v) with pH adjusted to 7.4 with HEPES was used in the D/P system.

2.2.3. Dissolution profile of drugs in the apical side of the D/P system

The dissolution profile of drugs was observed by using only the apical chamber of the D/P system. A flat sheet of aluminum foil was mounted between the chambers to prevent the leakage of apical solution to the basal compartment. An appropriate amount of crushed tablets of celecoxib (1, 4, 8 mg), montelukast sodium (0.02, 0.1, 0.5 mg), and zafirlukast (0.1, 0.2, 0.4, 0.8 mg) was applied to the apical solution, and aliquots of samples (0.1 mL) were routinely taken from the apical solution over 2 h. All samples were immediately filtered through a cellulose acetate filter (pore size: 0.45 μm), and each filtrate (0.05 mL) was mixed with 0.45 mL of the solution consisting of water and acetonitrile (50/50) to prevent the dissolved drug from precipitating in the apical solution. This solution was used for the assay. All experiments were performed at 37 °C under stirring at 200 rpm with a magnetic stirrer.

2.2.4. Dissolution and permeation study using the D/P system

A solution (TM, pH adjusted to 6.5) and the basal medium were introduced to the apical and basal sides of the Caco-2 cell monolayer in the well, respectively. After preincubation for 20 min, the Caco-2 cell monolayer with support filter was mounted between the chambers of the D/P system. Then, the apical side of the Caco-2 cell monolayer was filled with the apical medium, $FaSSIF_{mod}$. The basal side was filled with the basal medium in all experiments. Each side of the monolayer was continually stirred at 200 rpm with a magnetic stirrer. An appropriate amount of crushed tablets of celecoxib (1, 4, 8 mg), montelukast sodium (0.02, 0.1, 0.5 mg), and zafirlukast (0.1, 0.2, 0.4, 0.8 mg) was applied to the apical solution. Zafirlukast (0.4 mg) as bulk and solution (40 mg/mL) with a solubilized agent, WellSolve, were applied to the apical side of the D/P system. After addition of each drug to the D/P system, aliquots of samples (0.2 mL) were routinely taken from the basal solution for 2 h. The volume of the basal solution was maintained by adding fresh medium. After completion of the experiment for zafirlukast as bulk and solution, the apical solution was immediately collected and filtered through a cellulose acetate filter. All apical samples (0.05 mL) were mixed with 0.45 mL of the solution consisting of water and acetonitrile (50/50). The transepithelial electric resistance (TEER) of the Caco-2 cell monolayer was checked before and after the experiment. All experiments were performed at 37 °C. Significant change in the TEER value between before and after experiment in all studies was not observed, indicating that all studies with Caco-2 cell monolayer could be properly performed (data not shown).

2.3. Animal study

All animal experiments were approved by the Ethical Review Committee of Setsunan University and were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985). Male Wistar rats ($n = 4$) weighing 200–250 g were deprived of food but given free access to water for 18 h before the experiments. The dose of zafirlukast and administered water volume were set to 0.8 mg/kg and 4 mL/kg, respectively, which are comparable to the levels applied clinically (40 mg/body and 250 mL/body). As different dosage forms of zafirlukast, bulk, crushed tablets, and solution (40 mg/mL) with WellSolve were used. An appropriate amount of each dosage form was immediately administered to rats after being suspended in water containing 0.5% methylcellulose (0.2 mg/mL). At pre-determined time points, blood samples were collected from the jugular vein. The blood samples were centrifuged, and plasma was kept at −30 °C before quantification of drug.