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Effect of Absorption Behavior of Solubilizers on Drug Dissolution in the Gastrointestinal Tract: Evaluation Based on *In Vivo* Luminal Concentration–Time Profile of Cilostazol, a Poorly Soluble Drug, and Solubilizers

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ABSTRACT

The purpose of this study was to evaluate the effect of absorption behavior of solubilizers on drug dissolution in the gastrointestinal tract. After oral administration of FITC–dextran (FD-10), a nonabsorbable marker, and cilostazol (CZ), a low-solubility drug, with or without solubilizers (dimethyl sulfoxide [DMSO], and D-α-tocopherol polyethylene glycol 1000 succinate [TPGS]), the *in vivo* rat luminal concentrations of these compounds were determined by direct sampling of residual water in each segment of the gastrointestinal tract. DMSO was rapidly absorbed and not detected in the middle small intestine. Conversely, the TPGS concentration increased by 1.5- and 2-fold relative to the initial dose concentration in the middle and lower small intestine, respectively, owing to condensation. Then, normalized area under the luminal concentration–time curve of solid CZ was calculated from the luminal concentration–time profiles of FD-10 and solid CZ to evaluate *in vivo* dissolution behavior of CZ. The dissolution of CZ was marked when administered with TPGS compared with that when administered with DMSO, especially in the lower small intestine. This clearly indicates that absorbability of solubilizers is one of the important factors in determining the solubilizing effect. These findings may be beneficial to development of oral lipophilic drugs.

Introduction

Up to 75% of new drug candidates are hydrophobic and poorly soluble in water.[1,2] Such physicochemical properties of drug candidates frequently lead to low bioavailability after oral administration[3] and account for several failures in the development of oral products.

To cope with this problem, many types of solubilizers, such as surfactants and cosolvents, are currently used in oral formulations of poorly soluble drugs, including solubility-enabling formulations, such as supersaturable and lipid-based formulations.[4,5] Solubilizers are also used for immediate release formulations.[6,7] However, the effect of solubilizers on *in vivo* absorption often differs from that observed in *in vitro* studies, such as dissolution test or solubility measurements.[8-10] Although solubility–permeability interplay and/or inhibition of transporters in the gastrointestinal (GI) tract have been reported for this erratic improvement by solubilizers,[11,12] the solubilizing mechanisms are not completely understood.

Biopharmaceutics Classification System (BCS) based on the aqueous solubility and intestinal permeability of drugs has been used for biowaiver of human bioequivalence studies for immediate

Abbreviations used: $AUC_{GI(drug)}$, area under the luminal concentration-time curve of drug; $AUC_{GI(FD-4)}$, area under the luminal concentration–time curve of FD-4; BCS, Biopharmaceutics Classification System; CZ, Cilostazol; TPGS, D-α-tocopherol polyethylene glycol 1000 succinate; DMSO, dimethyl sulfoxide; FD-10, FITC-dextran 10S; Fa, fraction of drug absorbed; GI, gastrointestinal; IR, immediate release; PEG400, polyethylene glycol 400.

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release formulations.[13-15] According to the EMA, FDA, and WHO regulatory guidelines, oral products of BCS class I drugs with high permeability and high solubility[16] are eligible for biowaiver, when the several criteria for the comparative *in vitro* dissolution testing are fulfilled.[13-15] It has been reported that the bioequivalence failure rate is generally low (<11%),[17] and in the International Pharmaceutical Federation biowaiver monographs, positive biowaiver recommendation is given for the BCS class I drugs.[18]

However, the situation is different for poorly soluble drugs. As for BCS class II drugs (high permeability and low solubility drugs),[16] a biowaiver is considered in the WHO guideline only. This is because the bioequivalence failure rate of BCS class II drugs is very high (54%).[17] It has been often discussed to extend BCS-based biowaiver using comparative *in vitro* dissolution testing to BCS class II drugs.[6,7,10,19,20] However, because different types of solubilizers used in original oral products are generally used for the development of generics of poorly soluble drugs,[6,7,19] understanding the reasons for the erratic effect of solubilizers on the intestinal dissolution and absorption of drugs is imperative to develop bioequivalent oral products.

The author previously reported that *in vivo* luminal concentration after oral administration of drugs dynamically fluctuate according to their intestinal permeability and fluid volume in each segment of the GI tract.[21,22] It is visible that the luminal concentration–time profiles of solubilizers after oral administration also varied depending on their physicochemical properties. Thus, fluctuation in the luminal concentration of solubilizers may have profound influence on improvement of drug dissolution in the GI tract.

In this study, *in vivo* concentration–time profile was monitored in the rat GI tract after the oral administration of cilostazol (CZ) suspension containing fluorescein isothiocyanate dextran 10S (FD-10) with or without the solubilizers. Then, the effect of absorption behavior of the solubilizers on CZ dissolution in the GI tract was evaluated based on pharmacokinetic analysis from the luminal FD-10 and CZ concentration–time profiles.

Materials and Methods

Materials

FD-10 (MW 10,000) was purchased from Sigma-Aldrich (St. Louis, MO). CZ and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) was obtained from Eastman Chemical Company. All the other reagents were of analytical grade.

Preparation of CZ Suspension Containing FD-10 With or Without Solubilizers

Three solutions were prepared; one solution contained only 0.5% methyl cellulose. DMSO 15% (vol/vol) (165.1 mg/mL) was added to 0.5% methyl cellulose to make the second solution. The third solution was prepared by adding TPGS 2% (wt/vol) (20 mg/mL) to 0.5% methyl cellulose. CZ (3 mg/mL) and FD-10 (0.8 mg/mL) were suspended in above solutions.

Solubility Measurement of CZ in Simulated GI Fluids

Fasted state simulated upper GI fluid of rats ($FaSSIF_{rat,\ upper}$) has been developed based on actual GI fluid physiology.[23,24] This simulated fluid contains 50 mM sodium taurocholate and 3.7 mM egg–lecithin in phosphate buffer adjusted to pH 7.0. Saturated solubility of CZ was measured in $FaSSIF_{rat,\ upper}$ with or without 15% DMSO and 2.0% TPGS. Excess CZ was suspended in each $FaSSIF_{rat,\ upper}$ and vortexed. Each sample was kept in an incubator at 37°C, under shaking. The resulting suspensions were filtered through 0.45-μm cellulose membranes (Minisart RC4; Sartorius, Goettingen, Germany). CZ in the supernatant was analyzed by HPLC.

In Vivo *Measurement of Luminal Concentrations of FD-10, CZ, DMSO, and TPGS in Each Segment of the GI Tract and Plasma Concentration of CZ*

All animal studies were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Committee for Animal Experiments of Hiroshima International University.

Male Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). After oral administration of 1 mL of 3 CZ suspension containing FD-10, rats weighing about 220-250 g were sacrificed at set times. Then, the abdomen was opened immediately and residual water was obtained from the stomach, middle small intestine (from a site 21-36 cm distal to the stomach), and lower small intestine (10 cm from the cecum), using a micropipette. Each fluid sample was then divided between 2 plastic tubes, one with and one without 0.45-μm hydrophilic polyvinylidene fluoride centrifugal filter (Ultrafree®; Millipore Corporation). The sample volumes were calculated by subtracting the tare weight of the tube from the weight of the tube with sample fluid, assuming the relative density of GI fluid equals 1. The sample in the tube without the filter was diluted with DMSO based on the calculated fluid volume to dissolve solid CZ completely. After a second dilution with either methanol or Tris buffer (pH 7.7), the total CZ (solid plus dissolved CZ) concentration and FD-10 concentration were determined, respectively. In the tubes with the centrifugal filter, the filtrate was diluted with methanol to determine the concentration of dissolved CZ in the GI tract. The samples were again diluted with methanol to quantify the luminal DMSO and TPGS concentrations. The solid CZ concentration was calculated by subtracting the dissolved CZ concentration from the total CZ concentration.

Blood samples (approximately 1 mL) were taken from the jugular vein at the same time as the GI fluid sampling. Plasma was obtained by centrifugation and de-proteinized using methanol. After centrifugation, the resulting supernatant was evaporated before resuspension in the HPLC mobile phase, and CZ concentration was determined using HPLC.

Calculation of Normalized Area Under the Luminal Concentration–Time Curve of Solid CZ (Normalized $AUC_{GI(solid\ CZ)}$) in Each Segment of the GI Tract

Normalized area under the luminal concentration–time curve of solid CZ (normalized $AUC_{GI(solid\ CZ)}$) was calculated by following equation.

$$\text{Normalized } AUC_{GI(solid,\ CZ)} = AUC_{GI(solid\ CZ)} \times \frac{AUC_{GI(FD-10,\ without\ solubilizer)}}{AUC_{GI(FD-10)}} \times \frac{1}{AUC_{GI(solid\ CZ,\ without\ solubilizer)}}$$

where $AUC_{GI(solid\ CZ)}$, $AUC_{GI(FD\text{-}10)}$, $AUC_{GI(solid\ CZ,\ without\ solubilizer)}$, and $AUC_{GI(FD\text{-}10,\ without\ solubilizer)}$ are the area under the luminal concentration–time curve of solid CZ and FD-10 in each segment of the GI tract after oral administration with or without solubilizers, respectively. The AUC_{GI} value is mainly influenced by fluid volume and drug amount that passed through each segment. Because