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In vitro–in vivo correlation for wet-milled tablet of poorly water-soluble cilostazol

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ABSTRACT

The purpose of the present study was to investigate oral bioavailability of an immediate release tablet containing wet-milled crystals of a poorly water-soluble drug, cilostazol, and to establish in vitro–in vivo correlation. Sub-micron sized cilostazol (median diameter: 0.26 μm) was successfully prepared using a beads-mill in water in the presence of a hydrophilic polymer and an anionic surfactant. The milled suspension was solidified with a sugar alcohol as a water-soluble carrier by spray-drying method. The co-precipitate was compressed into an immediate release tablet with common excipients. Oral bioavailability of the wet-milled cilostazol tablet in male beagle dogs was 13-fold higher than the hammer-milled commercial tablet in fasted condition. Food did not increase the oral bioavailability of the wet-milled tablet, while 4-fold increase was found for the commercial tablet. Irrespective to the bioavailability enhancement, in vitro dissolution rate of the wet-milled tablet was even slower than the commercial tablet by the compendial method (USP Apparatus 2). On the other hand, a good correlation was found between the dissolution profiles obtained by a flow-through cell method (USP Apparatus 4, closed-loop system without outlet filter) using a large volume of water and sodium lauryl sulfate (SLS) solution at the concentration lower than the critical micellar concentration (cmc) as dissolution media corresponding to the fasted and fed conditions, respectively.

1. Introduction

Increasing number of poorly water-soluble chemical entities is well recognized in recent drug discovery [1–3]. These compounds are often associated with bioavailability problem for oral formulations. This can be solved by various formulation techniques [4–16] that improve solubility and/or dissolution of such drugs, especially for Biopharmaceutics Classification System (BCS) Class 2 compounds [17]. Particle size reduction is a well-known technique to enhance dissolution rate of poorly water-soluble drugs by expanding the specific surface area for dissolution. Recent progress in formulation technologies has enabled to produce a drug substance in sub-micron size range. In particular, wet-milling technique using beads mill is one of the most efficient ways to generate sub-micron drug crystals to improve oral bioavailability [6–12]. Elimination of positive food effect on bioavailability is another advantage of the sub-micron drug particles [9–11]. Several commercial products, such as Rapamune® (sirolimus), Emend® (aprepitant), TriCol® (fenofibrate) and Megace® ES (megestrol), have been commercialized by using a proprietary wet-milling technology, NanoCrystal®. However, little is published about in vitro–in vivo correlation (IVIVC) of formulations containing sub-micron drug substances. This is probably because of the difficulties to develop appropriate dissolution test methods due to extremely fast dissolution of sub-micron drug substances and to separate undissolved particles from dissolution medium. A few attempts were reported in the literature to overcome the issues by utilizing centrifugation [6], dialysis [18] and turbidimetric analysis [19].

Previously, we reported the effect of particle size on the dissolution and oral absorption of a poorly water-soluble anti-platelet aggregation agent, cilostazol [11]. Cilostazol can be categorized into BCS class 2 (low solubility–high permeability), based on the low aqueous solubility (6 μg/mL at 37 °C) [20] and a relatively high apparent permeability coefficient in Caco-2 cell monolayer (2×10^{-6} cm/s) [21]. In the study, hammer-milled, jet-milled and wet-milled cilostazol having the mean diameters of 13, 2 and 0.2 μm, respectively, were investigated as aqueous suspensions. Dissolution tests were performed for the small amount of cilostazol in water with USP Apparatus 2. The dissolution rates of the wet-milled cilostazol suspension as well as the others were well described by the model developed by Hintz et al. [22–23]. The in vitro dissolution rate and bioavailability of cilostazol in beagle dogs were significantly increased according to the particle size reduction. Positive food effect on bioavailability was observed for the hammer-milled and jet-milled suspensions in dogs as it was reported for the commercial formulation in humans [24]. The wet-milled suspension, however, exhibited no significant food effect, which would be attributed to the maximization of dissolution rate [11].

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In the present study, the potential on bioavailability enhancement of the wet-milled cilostazol was further investigated by formulating into an immediate release tablet. Furthermore, the in vitro dissolution method reflecting the in vivo absorption kinetics was established.

2. Material and methods

2.1. Material

Cilostazol and its commercial formulation (Pletal® 100-mg tablet, prepared with hammer-milled cilostazol) were obtained from Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). An internal standard OPC-13012 (6-[4-(1-cyclohexyl- 1H- tetrazol-5-yl) propoxy]-3,4-dihydro-1-ethyl-2(1H)-quinolinone) was synthesized in Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Hydroxypropyl cellulose (HPC) was purchased from Nippon Soda Co., Ltd. (HPC-SL grade, Tokyo, Japan). Docusate sodium (DOSS) was purchased from Sigma-Ardrich Japan Co., Ltd. (Tokyo) and sodium lauryl sulfate (SLS) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). D-mannitol was obtained from Roquette Japan (Tokyo). Corn starch and croscarmellose sodium were obtained from Nihon Shokuhin Kako Co., Ltd. (Tokyo) and Asahi Kasei Corporation (Tokyo), respectively. Polysorbate 80 (PS80) was purchased from NOF Corporation (HM grade, Tokyo). Sodium taurocholate and egg lecithin (biochemistry grade) were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo) and Kanto Chemical Co., Inc. (Tokyo), respectively.

2.2. Hammer-milled and jet-milled cilostazol

The hammer-milled cilostazol crystal was prepared with Atomizer AII W5G (Dalton, Tokyo) and the jet-milled cilostazol crystal was prepared with Super Sonic Jet Mill PJM-100SP (Nippon Pneumatic MFG Co., Ltd. Osaka). The median and 90% diameters of the hammer-milled cilostazol crystal were 13 and 44 μm, respectively, and those of the jet-milled crystal were 2.4 and 5.2 μm, respectively.

2.3. Wet-milled cilostazol

Aqueous suspension consisting of 82.7% cilostazol, 16.5% HPC and 0.8% DOSS was prepared at the total solid content of 10%. HPC and DOSS were added as steric and charge stabilizers, respectively, so as to prevent aggregation of the milled particles. The suspension was grounded with Dyno-Mill (KDL Type-A, Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland) filled with 0.2 mm i.d. zirconium beads for 270 min with periodically monitoring the particle size. D-mannitol was added to the milled suspension at the equivalent weight to cilostazol, and then the mixture was solidified with a spray drier (GB-21, Yamato Science Co., Ltd., Tokyo). The yielded powder exhibited excellent re-dispersibility in water. The crystal form and chemical purity of cilostazol were not changed by the treatments. Median and 90% diameters of the milled cilostazol crystal were found to be 0.26 and 0.61 μm, respectively.

2.4. Wet-milled cilostazol tablet

The wet-milled cilostazol spray dried powder co-precipitated with D-mannitol was compressed into a 100-mg tablet with corn starch and croscarmellose sodium using a universal tester (Autograph AG-I 50KN, Shimadzu, Kyoto, Japan) equipped with a 13.0 mm i.d. tooling at 15 KN. D-mannitol added to the formulation was found to be essential to acquire good re-dispersibility of the sub-micron cilostazol crystals when the tablet disintegrated in aqueous media (data not shown). A similar result was reported for nanocrystals of nifedipine [25].

2.5. Simulated intestinal fluid in the fed state (FeSSIF)

The simulated intestinal fluid in the fed state (FeSSIF) was prepared of 15 mM of sodium taurocholate and 3.75 mM lecithin, adjusted to pH 5.0 [26].

2.6. Particle size distribution measurement

Particle size distributions of the milled cilostazol crystals were determined with a laser diffraction particle size analyzer, SALD-3000J (Shimadzu, Kyoto), in 0.5% hydroxypropyl methylcellulose aqueous solution as a dispersing medium.

2.7. Solubility measurement

Equilibrium solubility values of cilostazol at 37 °C were determined in 0.30% SLS, of which the use is regulated to assure the dissolution property of commercial tablets of cilostazol [27], and 4.0% PS80. Excess amount of the jet-milled cilostazol crystal was added in each medium in a screw-capped vial. Then, the vials were shaken continuously in a water bath maintained at 37 °C for 24 h. The equilibrated samples were immediately filtered through a 0.2 μm membrane filter, and the filtrate was diluted with an appropriate volume of methanol. A 50 μL volume of the sample was analyzed by a reversed phase HPLC method described below.

Solubility samples were introduced onto an HPLC system (Model LC-2010, Shimadzu) with a detection wave-length of 254 nm. A C18 column (TSK gel ODS-80Ts, 4.6 mm i.d.×150 mm, Tosoh Co., Ltd., Tokyo) was used as an analytical column. A mobile phase containing 0.2w/v% SLS and 0.03v/v% phosphoric acid in acetonitrile–methanol–water mixture (3:3:4, v/v) was delivered at 1.0 mL/min. The correlation coefficient of standard curve from 2.0 to 100.8 ng/mL was over 0.999 and the coefficient of variation of standard curve ranged from 0.03 to 0.45%. The equilibrium solubilities of cilostazol in 0.30% SLS and 4.0% PS80 were determined to be 154±4 and 110±1 μg/mL at 37 °C, respectively.

2.8. Dissolution test (USP Apparatus 2)

The compendial dissolution test method, USP Apparatus 2, was employed at a paddle rotational speed of 50 rpm using 900 mL of 0.30% SLS [27], 4.0% PS80 or water as the dissolution medium. The dissolution tests were performed using DT-610 dissolution tester (JASCO, Tokyo) connected with a UV spectrophotometer. Dissolved cilostazol was quantified from absorbance difference between the wave-lengths of 257 nm and 325 nm.

2.9. Dissolution test (USP Apparatus 4)

A flow-through cell dissolution tester (PT-DZ7, Pharma Test Apparatebou GmbH, Hainburg, Germany) was used in two different flow patterns illustrated in Fig. 1(A) and (B) as the open and closed-loop settings in this research, respectively. A ruby ball was placed on the fluid inlet orifice of the dissolution cell (22.6 mm i.d.) to prevent backflow and glass beads were placed on the bottom cone of the cell to generate a laminar flow. The sample tablet was placed in the middle of the cell with a tablet holder. The dissolution medium was placed in a temperature-controlled reservoir of 20 L capacity (TS-200, Toyama Sangyo Co., Ltd., Osaka).

In the open-loop setting, the dissolution medium is pumped out from the dissolution cell through an outlet filter (cut-off size 0.3 μm, Gamma 12 filter, Grade 03, Whatman Japan KK, Tokyo), lead to a fraction collector. In-line assay was not feasible since the medium out of the cell was strongly turbid because of sub-micron particles that could be passed through the outlet filter. Therefore, the collected samples were centrifuged at 25,000 ×*g* for 20 min so as to remove