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In vivo evaluation of supersaturation/precipitation/re-dissolution behavior of cinnarizine, a lipophilic weak base, in the gastrointestinal tract: the key process of oral absorption

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ABSTRACT

The aim of this study is to evaluate how supersaturation, precipitation, and re-dissolution processes influence the intestinal absorption of cinnarizine (CNZ), a lipophilic weak base, by monitoring its plasma and luminal concentration–time profile, after oral administration as a HCl solution containing fluorescein isothiocyanate dextran (FD-4), a non-absorbable marker. In the *in vitro* pH shift experiment, the supersaturation stability was significantly lower when the higher-concentration solution of CNZ (pH 1.5) was added to the simulated intestinal fluid. However, although the *in vivo* bioavailability after oral administration of high and low dose as HCl solutions was greatly improved compared to those as neutral suspensions, the difference in the supersaturation stability was not reflected in the improvement of the *in vivo* bioavailability. Analysis of CNZ and FD-4 concentrations in each segment of the gastrointestinal tract revealed that most of the CNZ precipitated in the duodenum after gastric emptying, and supersaturation was observed only in the duodenum. Thereafter, the precipitate was rapidly re-dissolved and absorbed in the upper and middle small intestine. The rapid re-dissolution may be caused by smaller particles of the precipitate. In this case, it is considered that the key process for the absorption of CNZ was re-dissolution, not supersaturation. Therefore, different supersaturation stabilities in different doses observed in *in vitro* precipitation experiment was not reflected to *in vivo* absorption. These findings may be useful to design efficient supersaturable formulations and to validate and improve current prediction methods.

1. Introduction

Supersaturation can be created by using supersaturable formulations such as salts, cocrystals, and solid dispersions (Terebetski et al., 2014; Childs et al., 2013; Jackson et al., 2016). They may occur when lipophilic weak bases enter the neutral condition in the small intestine from the acidic condition in the stomach (Mitra and Fadda, 2014). Since supersaturation is thermodynamically unstable, the supersaturated drug concentration generally decreases to the equilibrium solubility due to precipitation. Supersaturation/precipitation processes are greatly influenced by many physiological factors including the pH, gastric emptying rate, and components of the intestinal fluid (Bevernage et al., 2010; Mitra and Fadda, 2014), and therefore, sometimes causes large intra- and inter-individual variabilities in the drug dissolution and absorption.

To avoid failures in the development of basic drugs or supersaturable formulations, it is very important to evaluate the oral absorption as early as possible, before beginning clinical studies. Currently, there are many reports regarding prediction methods for the oral absorption of such drugs and formulations using the physiologically based pharmacokinetic model or predictive *in vitro* dissolution instruments (Wagner et al., 2012; Matsui et al., 2015). However, there are some cases where the *in vivo* outcomes, like the drug plasma exposure following oral administration of a supersaturable formulation, differ considerably from those predicted by the *in vitro* experiment (Carlert et al., 2010).

It is well known that an increasing degree of supersaturation of drugs accelerates subsequent precipitation (decreases supersaturation stability) (Bevernage et al., 2010, 2012; Ueda et al., 2014). Therefore, non-linearity in intestinal absorption is sometimes observed after administration of supersaturable formulation at various doses (Psachoulias et al., 2012; Knopp et al., 2016). Conversely, there is a report that the supersaturation caused linear absorption kinetics regardless of dose. (Carlert et al., 2010). Therefore, understanding what happens in the gastrointestinal (GI) tract after the oral administration of drugs

Abbreviations: $AUC_{duodenum}$, area under the duodenal concentration-time curve; BA, bioavailability; BCS, Biopharmaceutics Classification System; CNZ, cinnarizine; DSC, differential scanning calorimetry; FD-4, FITC-dextran; GI, gastrointestinal; HPLC, high-performance liquid chromatography.

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showing supersaturation is of significance when designing efficient formulations, in addition to validating and improving prediction methods.

Sugita et al. (2014) has reported that since the particle size of pioglitazone, a low solubility drug, fluctuated after precipitation from the supersaturated state, the re-dissolution process is important to develop its bioequivalent product of salt formulation. It has also been reported that the crystal transformation from stable to the meta-stable form of a precipitated drug had a great impact on the *in vivo* oral absorption (Hisada et al., 2016). This re-dissolution process of precipitates makes their absorption behavior more complicated. However, information about analyzing *in vivo* re-dissolution behavior in the GI tract is very limited.

In this study, an *in vivo* concentration-time profile was monitored in the rat GI tract after oral administration of high and low doses of cinnarizine (CNZ), a lipophilic weak base, as a HCl solution containing fluorescein isothiocyanate dextran (FD-4, MW4000), a non-absorbable marker. Then, we evaluated the key process for improvement of intestinal absorption of supersaturated CNZ through the comparison of luminal FD-4 and CNZ concentrations.

2. Materials and methods

2.1. Materials

FD-4, a non-absorbable marker, and pentagastrin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). CNZ was purchased from LKT Laboratories Inc. (St. Paul, Minnesota, USA). All the other reagents were of analytical grade.

2.2. Preparation of CNZ solution (pH 1.5)

It was expected that a substantial amount of time (more than approximately 5 min) is required to completely dissolve CNZ in HCl (pH 1.5) for preparation of high-concentration solution (1694 and 3080 μg/mL). Hence, first, excess CNZ was suspended in HCl (pH 1.5) and vortexed at room temperature. Then, the suspension was filtered using a 0.45 μm MS® PTFE Syringe Filter (Membrane Solutions Limited, Dallas, Texas, USA) to prepare the saturated CNZ solution (the saturated solubility of CNZ was 5075 μg/mL). The solution was diluted with HCl (pH 1.5) to make CNZ solutions (pH 1.5) with various concentrations.

2.3. Solubility measurement of CNZ in simulated GI fluids

A fasted state simulated upper GI fluid of rats ($FaSSIF_{rat,\ upper}$) has been developed based on actual GI fluid physiology (Tanaka et al., 2012, 2014). Briefly, an isotonic phosphate buffer (pH 7.0) was prepared by mixing 2.54% $NaH_2PO_4 \cdot 2H_2O$ and 4.41% $Na_2HPO_4 \cdot 12H_2O$. Then, distilled water was added to the phosphate buffer solution in the ratio of 4:16. Sodium taurocholate and egg-lecithin were dissolved in the phosphate buffer at a concentration of 50 mM and 3.7 mM, respectively, to make the $FaSSIF_{rat,\ upper}$.

Excess CNZ was suspended in the $FaSSIF_{rat,\ upper}$ and vortexed. Each sample was kept in an incubator at 37 °C for about 24 h. The suspensions were filtered through 0.45 μm cellulose membranes (Minisart RC4, Sartorius Japan, Tokyo, Japan). CNZ in the filtrate was analyzed by high-performance liquid chromatography (HPLC). Solubility of CNZ in various medium are summarized in Table 1.

2.4. In vitro miniscale dissolution test to evaluate the supersaturation/precipitation behavior of CNZ

Pre-$FaSSIF_{rat,\ upper}$ was prepared by dissolving sodium taurocholate (57.7 mM) and egg-lecithin (4.27 mM) in phosphate buffer (pH 7.2). $FaSSIF_{rat,\ upper}$ can be made by mixing Pre-$FaSSIF_{rat,\ upper}$ with HCl (pH 1.5) at a ratio of 13:2, although the phosphate concentration (50 mM) is different from original $FaSSIF_{rat,\ upper}$. However, the saturated solubility of CNZ in the modified $FaSSIF_{rat,\ upper}$ was almost same as that in original, indicating that the two simulated intestinal fluids have the same solubilizing capacity.

In vitro supersaturation/precipitation behavior of CNZ was evaluated by the paddle method (50 rpm, 37 °C) using Dissolution Apparatus (708-DS, Agilent Technologies, Santa Clara, California, USA). Six milliliters of HCl (pH 1.5), dissolving CNZ at high (3080 μg/mL) and low (656 μg/mL) concentrations, was added to 39 mL of the Pre-$FaSSIF_{rat,\ upper}$ in a 100 mL vessel. Then, samples of about 1 mL were taken at each time point and instantly filtered through 0.45 μm cellulose membranes, and the filtrates were diluted with 40% acetonitrile for CNZ quantification.

2.5. In vivo measurement of the plasma concentration of CNZ and luminal concentrations of FD-4 and CNZ in each segment of the GI tract

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 the Hiroshima International University.

High (1694 ± 244 μg/mL) and low (372 ± 52 μg/mL) concentrations of CNZ solutions (pH 1.5) containing FD-4 (200 μM) were prepared. Since the CNZ solution was produced prior to every animal experiment, there was some variability in the concentration.

To make pentagastrin solution (66.7 μg/mL), pentagastrin was dissolved in saline (pH 11), and then the solution was diluted with isosmotic phosphate buffer (pH 7.0).

The pentagastrin solution (0.3 mL) and HCl (0.5 mL, pH 1.8) were intraperitoneally and orally administered to fasted Male Wistar rats (Japan SLC, Shizuoka, Japan) weighing about 200–220 g about 10–20 min and 1 min before oral administration of 1 mL of the two CNZ solutions, respectively. The administration of pentagastrin and HCl was to maintain low pH in the stomach. Then, blood samples (about 1 mL) were taken from the jugular vein, under anesthesia by diethyl ether, and the rats were sacrificed at 5, 10, 15, 20, 30, 50 and 70 min. Thereafter, the abdomen was opened immediately to collect residual fluid from the stomach, duodenum, upper small intestine (about 20 cm range from the Treitz ligament), and middle small intestine (20–35 cm range from Treitz ligament). After fluid sampling, the gastric pH was measured by directly inserting a pH spear (Nikko Hansen & Co., Ltd., Osaka, Japan) into the stomach.

Plasma obtained by centrifugation was de-proteinized by methanol precipitation. After centrifugation, the CNZ in the resulting supernatant was determined using LC-MS/MS.

The processing of intestinal fluid samples was conducted in almost the same way reported in our previous work (Tanaka et al., 2016). Briefly, obtained fluid samples were divided between two plastic tubes, one with and one without a 0.45 μm hydrophilic polypropylene centrifugal filter (GHP Nanosep® MF Centrifugal Device, Pall Corporation, Port Washington, New York, USA). The difference between the tare mass of the tube and the mass of the tube with sample fluid was regarded as the sample volume, assuming the relative density of GI fluid equals 1. The sample in the tube without the filter was diluted with DMSO containing 1% of 1 M Tris buffer (pH 7.7) based on the calculated fluid volume to fully dissolve the solid CNZ. After a second dilution with either 50% DMSO or 50 mM Tris buffer (pH 7.7), the total CNZ concentration (solid plus dissolved CNZ) and FD-4 concentration were quantified by HPLC and multilabel luminescence counter, respectively. In the tubes with the centrifugal filter, the filtrate was diluted with 50% DMSO

Table 1
Solubility of CNZ in various medium ($n = 3$–4).

	Solubility (μg/mL)
Water	0.015 ± 0.003
HCl (pH 1.5)	5075 ± 151
$FaSSIF_{rat,\ upper}$	12.15 ± 0.16