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In vivo analysis of supersaturation/precipitation/absorption behavior after oral administration of pioglitazone hydrochloride salt; determinant site of oral absorption

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ABSTRACT

The purpose of this study was to evaluate *in vivo* supersaturation/precipitation/absorption behavior in the gastrointestinal (GI) tract based on the luminal concentration-time profiles after oral administration of pioglitazone (PG, a highly permeable lipophilic base) and its hydrochloride salt (PG-HCl) to rats. In the *in vitro* precipitation experiment in the classic closed system, while the supersaturation was stable in the simulated gastric condition, PG drastically precipitated in the simulated intestinal condition, particularly at a higher initial degree of supersaturation. Nonetheless, a drastic and moderate improvement in absorption was observed *in vivo* at a low and high dose of PG-HCl, respectively. Analysis based on the luminal concentration of PG after oral administration of PG-HCl at a low dose revealed that most of the dissolved PG emptied from the stomach was rapidly absorbed before its precipitation in the duodenum. At a high dose of PG-HCl, PG partly precipitated in the duodenum but was absorbed to some extent. Therefore, the extent of the absorption was mainly dependent on the duodenal precipitation behavior. Furthermore, a higher-than expected absorption after oral administration of PG-HCl from *in vitro* precipitation study may be due to the absorption process in the small intestine, which suppresses the precipitation by removal of the drug. This study successfully clarify the impact of the absorption process on the supersaturation/precipitation/absorption behavior and key absorption site for a salt formulation of a highly permeable lipophilic base based on the direct observation of *in vivo* luminal concentration. Our findings may be beneficial in developing an ideal physiologically based pharmacokinetic model and *in vitro* predictive dissolution tools and/or translating the *in silico* and *in vitro* data to the *in vivo* outcome.

1. Introduction

Many of the drug candidates identified in drug discovery studies are poorly soluble in water (Williams et al., 2013). Such physicochemical property often results in low and variable oral absorption (Williams et al., 2013). Supersaturation of a drug in the gastrointestinal (GI) tract is considered an efficient method to overcome this problem, and can be achieved through development of supersaturable formulations such as salts, co-crystals, and solid dispersions (Terebetski et al., 2014; Aher et al., 2010; Knopp et al., 2016). Furthermore, the pH gradient between the stomach and duodenum may induce supersaturation of a lipophilic base (Tsume et al., 2017).

Supersaturated drug is thermodynamically unstable, which leads to drug precipitation in the GI tract (Bevernage et al., 2011). The precipitation rate is generally accelerated with increasing supersaturated drug concentration (Ueda et al., 2014), sometimes inducing non-linear absorption after oral administration of supersaturable formulations (Psachoulias et al., 2012; Knopp et al., 2016).

To date, various *in vitro* predictive dissolution models, such as an artificial stomach-duodenum dissolution model (Lee et al., 2017), *in vitro* dissolution test with permeation bag (Hens et al., 2015), dissolution/permeation system (Kataoka et al., 2006), and GI simulator (Takeuchi et al., 2014; Matsui et al., 2016) have been developed; biorelevant media are generally used for these methodologies. There

Abbreviations: (AUC_{GI}), Area under the luminal concentration-time curve; (BA), bioavailability; (DS), degree of supersaturation; ($FaSSIF_{rat,\ upper}$), Fasted state simulated upper intestinal fluid of rats; (FD-4), FITC-dextran; (GI), gastrointestinal; (HPLC), high-performance liquid chromatography; (PB), phosphate buffer; (PG), pioglitazone; (PG-HCl), pioglitazone hydrochloride salt

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are considerable regional differences in physiological parameters, such as fluid volume, pH, and components of the GI fluid (Masaoka et al., 2006; Tanaka et al., 2012), and these physiological parameters greatly influence supersaturation/precipitation behavior of a drug (Bevernage et al., 2011, 2012a; Ruff et al., 2017). Hence, the actual supersaturation/precipitation behavior may be greatly dependent on the site of the GI tract. To know the sites at which GI tract absorption and precipitation of supersaturated drug mainly occur may be of significance in selection and creation of ideal *in vitro* dissolution methodologies, biorelevant media, and/or physiologically based pharmacokinetic (PBPK) model.

Bevernage et al. (2012b) reported that precipitation of loviride, a low-solubility drug, in an *in vitro* precipitation assessment with absorption environment was less extensive than that with non-absorption environment. They asserted the importance of the absorption process in the supersaturation/precipitation behavior. However, the impact of intestinal absorption process on supersaturation/precipitation behavior of drugs in the GI tract after the oral administration of supersaturable formulations remains unclear.

In this study, pioglitazone (PG) and its hydrochloride salt (PG-HCl) were administered as suspensions with fluorescein isothiocyanate dextran (FD-4, MW4000), a non-absorbable marker, to rats. PG is a highly permeable lipophilic base (Takagi et al., 2006) with MW = 356.45, log P = 2.3 (Drug Delivery Foundation, BCS Database), and pKa = 5.8 and 6.4 (Drug information provided by Takeda pharmaceutical Co., Ltd.). After oral administration, the luminal concentrations of PG and FD-4 were determined by direct GI fluid sampling. Furthermore, the normalized area under the luminal concentration–time curve (normalized AUC_{GI}) of PG was calculated based on the luminal concentrations, according to our previous study (Tanaka et al., 2016), to analyze the intestinal supersaturation/precipitation/absorption behavior of PG-HCl at different doses.

2. Materials and methods

2.1. Materials

PG and PG-HCl were obtained from Sawai Pharmaceutical Co., Ltd. (Osaka, Japan). FD-4 was purchased from Sigma-Aldrich (St. Louis, Missouri). All other reagents were analytical grade commercial products.

2.2. Preparation of various PG and PG-HCl suspensions

PG powder was gently grinded using a mortar and pestle to crush large aggregates. The morphology of the grinded PG and PG-HCl particles observed by a scanning electron microscope (JSM-6510A, JEOL Ltd., Tokyo, Japan) was a needle-like and block-like structure, respectively, with particle sizes of 10–50 μm.

PG-HCl (0.5 or 3 mg/mL as free form) was suspended in 0.5% methylcellulose solution. These suspensions were used for *in vitro* pH-shift experiment (Section 2.4).

For animal study (Section 2.5), FD-4 was dissolved in the two PG-HCl suspensions at a concentration of 200 μM. As a reference, PG (0.5 mg/mL) suspension containing FD-4 (200 μM) was prepared.

2.3. Solubility measurement

A fasted-state simulated upper intestinal fluid of rats ($FaSSIF_{rat,\ upper}$) was prepared by dissolving 50 mM sodium taurocholate and 3.7 mM egg-lecithin in 50 mM phosphate buffer (PB, pH 7.0) (Tanaka et al., 2017). An excess of PG was added in the $FaSSIF_{rat,\ upper}$ and 50 mM PB (pH 2.4). These suspensions were vortexed and shaken in an incubator for 3 h at 37 °C. $FaSSIF_{rat,\ upper}$ samples were filtered through a 0.45-μm cellulose membrane filter (Minisart RC4, Sartorius, Goettingen, Germany) and 50 mM PB (pH 2.4) samples were filtered through 0.45-μm hydrophilic polypropylene centrifugal filter (GHP Nanosep® MF Centrifugal Device, Pall Corporation, New York, USA). The concentrations of PG in these filtrates were quantified using high-performance liquid chromatography (HPLC).

2.4. In vitro precipitation behavior of PG after pH shift

As soon as PG-HCl (0.5 or 3 mg/mL as free form) suspensions were prepared, 1.5 mL of 250 mM PB (pH 2.4) or Pre-$FaSSIF_{rat,\ upper}$ (250 mM PB (pH 7.0), containing 250 mM sodium taurocholate and 18.5 mM egg-lecithin) were added to 6.0 mL of the two PG-HCl suspensions to mimic the rat gastric and intestinal conditions, respectively. In this case, the test medium was almost made up to 50 mM PB (pH 2.4) or $FaSSIF_{rat,\ upper}$, and the total PG concentrations in these media (80% of the initial concentration) almost captured the *in vivo* gastric concentration at 0 min (73.4% of the administered concentration) and the maximum duodenal concentration (84.7% of the administered concentration) of the total PG after the oral administration of the PG suspension (Section 3.3). The pH 2.4 was referred to as gastric pH when 0.5% methylcellulose solution was administered to rats at a dose of 5 mL/kg (Kosugi et al., 2015). *In vitro* precipitation experiment was conducted using a glass vial and magnetic stirrer at 150 rpm and 37 °C in an incubator. After pH shift, samples were withdrawn at designated times and filtered using a 0.45-μm cellulose membrane filter for neutral samples ($FaSSIF_{rat,\ upper}$) and a 0.45-μm hydrophilic polypropylene centrifugal filter for acidic samples [50 mM PB (pH 2.4)]. The concentrations of PG in these filtrates were quantified using HPLC.

2.5. In vivo evaluation of GI concentrations of FD-4 and PG and plasma concentration of PG

Male Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). 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.

Blood and GI fluid sampling was conducted similar to that in our previous study (Tanaka et al., 2016, 2017). Immediately after preparation of the three suspensions (Section 2.2), 1 mL each was intragastrically administered by oral gavage to the fasted rats weighing 200–220 g (the dose was 0.5 mg/body for PG and 0.5 or 3 mg/body as a free form for PG-HCl). Thereafter, blood samples (~1 mL) were collected at designated time points from the jugular vein under anesthesia by diethyl ether. Then, the rats were sacrificed and their abdomen was opened immediately to collect residual fluid from the stomach, duodenum, upper small intestine (~20 cm from the Treitz ligament), and middle small intestine (25–40 cm from Treitz ligament).

Blood samples were centrifuged, and the plasma obtained was deproteinized by methanol precipitation. After centrifugation, PG in the resulting supernatant was quantified by LC-MS/MS.

A portion of fluid sample was collected in a plastic tube, and the rest was collected in a tube with a 0.45-μm hydrophilic polypropylene centrifugal filter and filtered. The fluid volume was calculated by subtracting tare weight of the tube from the weight of the tube with sample fluid, assuming the relative density of GI fluid was equal to 1. The sample in the plastic tube was diluted with DMSO based on the calculated fluid volume to completely dissolve the solid PG (solid PG derived from PG-HCl also may be partly included, if PG-HCl was not fully dissolved in the GI tract). After a second dilution with 55% methanol with 17% polyethylene glycol 400 or 50 mM Tris buffer (pH 7.7), the total PG concentration (solid plus dissolved PG) and FD-4 concentration were quantified, respectively. In the tubes with the centrifugal filter, the filtrate was diluted with 55% methanol with 17% polyethylene glycol 400 to quantify the concentration of dissolved PG in the fluid samples. The solid PG concentration was calculated by subtracting the dissolved PG concentration from the total PG