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The Evaluation of *In Vitro* Drug Dissolution of Commercially Available Oral Dosage Forms for Itraconazole in Gastrointestinal Simulator With Biorelevant Media

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ABSTRACT

The purpose of this study was to assess the feasibility of a multicompartmental *in vitro* dissolution apparatus, gastrointestinal simulator (GIS), in assessing the drug dissolution of 2 commercially available oral dosage forms for itraconazole (ICZ). The GIS consists of 3 chambers, mimicking the upper gastrointestinal tract. *In vitro* dissolution of ICZ capsule or oral solution was evaluated in United States Pharmacopeia apparatus II and GIS. To investigate the suitability of fasted state simulated intestinal fluid (FaSSIF) to predict better *in vivo*, FaSSIF as well as phosphate buffer were used as dissolution media. Area under the dissolved drug amount-time curve (AUDC) was calculated for each dosage form in each apparatus, and the ratios of $AUDC_{oral\ solution}$ to $AUDC_{capsule}$ were compared with human pharmacokinetic data. Based on this comparison, GIS with FaSSIF can adequately distinguish the pharmacokinetic profiles of 2 oral dosage forms for ICZ. Additionally, Caco-2 cell transepithelial transport study in combination with GIS revealed that improved drug dissolution by formulations resulted in enhanced permeation of ICZ through cell monolayer, suggesting the observed ICZ concentration in the GIS will directly reflect systemic exposure. These results indicate GIS would be a powerful tool to assess the formulations of ICZ as well as other Biopharmaceutics Classification System class II drug formulations.

Introduction

Poor solubility of oral drug products is a growing concern in drug discovery and development. Due to recently introduced combinatorial chemistry, high-throughput screening and structure-based drug design, potential drug candidates tend to be more lipophilic.[1] Because low drug solubility causes several issues such as poor bioavailability and individual variability in drug exposure, oral formulation strategies have been widely adopted to improve drug solubility in many pharmaceutical industries.[2] However, formulation development has often been misled when those formulations for low-soluble drugs are assessed in conventional *in vitro* dissolution tests using United States Pharmacopeia (USP) apparatus I and II.[3,4] It is because these dissolution tests use a constant fluid volume, pH, and buffer species, which are not physiologically relevant in human gastrointestinal (GI) tract. As a result, it is difficult to predict *in vivo* performance of oral drug products and to obtain good *in vitro*–*in vivo* correlation. To assure the quality of oral formulation, *in vivo* predictive dissolution methodologies which incorporate dynamic physiological factors in the GI tract should be proposed.

Several *in vivo* predictive dissolution methodologies have been designed to improve *in vitro*–*in vivo* correlation.[5] Gastrointestinal simulator (GIS) is one of the most prominent *in vitro* dissolution apparatuses in evaluating the dissolution of certain drugs. GIS consists of 3 chambers, representing the stomach, duodenum, and

Abbreviations: AUC, area under the curve; AUDC, area under the dissolved drug amount-time curve; BCS, Biopharmaceutics Classification System; BSA, bovine serum albumin; EMA, European Medicines Agency; FaSSIF, fasted state simulated intestinal fluid; FDA, Food and Drug Administration; GIS, gastrointestinal simulator; GI, gastrointestinal; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HP-β-CD, hydroxypropyl beta cyclodextrin; ICZ, itraconazole; NaCl, sodium chloride; NaTC, sodium taurocholate; PTFE, polytetrafluoroethylene; PVDF, polyvinylidene difluoride; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; TEER, transepithelial electrical resistance; USP, United States Pharmacopeia.

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proximal jejunum.[6-9] As previously reported by Takeuchi et al.,[6] the physiological gastric transfer rate of GIS has been adjusted to fit *in vivo* pharmacokinetic profiles of Biopharmaceutics Classification System (BCS) class I drugs in fasted state. It was also revealed that the supersaturation and precipitation of dasatinib, a typical poorly soluble drug with weak base property, was observed in the GIS.[8] In addition, the GIS was able to capture the potential reduction in bioavailability of dipyridamole, also a weak base drug, caused by elevated gastric pH.[9] Dipyridamole has high aqueous solubility in the acidic stomach but exhibits low solubility in neutral pH condition, which is represented in the duodenum and jejunum. In fact, human intubation study revealed that dipyridamole exhibited higher drug concentrations than its equilibrium solubility in human GI tract, suggesting the observation of supersaturation in *in vivo*.[10] Because GIS adequately mimicked its higher intraluminal drug concentration, GIS is a promising apparatus to assess the *in vivo* dissolution of weak base drugs. With current needs for dosage assessment, it is of interest whether the GIS is suitable to evaluate the dosage form of certain drugs which have pH-dependent solubility.

In this study, itraconazole (ICZ) was selected as a test drug. ICZ is a triazole antifungal agent and is classified into BCS class II (high permeability, low solubility, Log *P* 5.66 at pH 8.1).[11,12] ICZ has a weak base property (pKa; 3.7) and, thus, exhibits pH-dependent solubility in physiological pH range.[13] The aqueous solubility of ICZ is less than 1 ng/mL at neutral pH and 4 μg/mL in 0.1-N HCl.[14] As ICZ itself is hardly bioavailable, there are 2 commercially available formulations for ICZ, a capsule and an oral solution.[15] A capsule formulation contains amorphous ICZ, whereas oral solution dosage form is produced by solubilized ICZ with hydroxypropyl-β-cyclodextrin (HP-β-CD) in an oral solution.[14-17] Because these formulations use different technologies, they exhibit distinct pharmacokinetic profiles in human.[16]

To evaluate *in vitro* dissolution of these 2 dosage forms, an ICZ 100-mg capsule or ICZ 10-mL oral solution (10 mg/mL, 100 mg) was dosed in 2 different dissolution apparatuses, USP apparatus II and GIS. Drug amount in solution-time profiles were obtained and compared with human pharmacokinetic data after a single dose of ICZ 100 mg in fasted state. In the previous GIS dissolution studies, 50-mM phosphate buffer at pH 6.5 ($SIF_{pH6.5}$) was used as a duodenal buffer.[6-9] However, it has been reported that fasted state simulated intestinal fluid (FaSSIF) predicts better *in vivo* dissolution.[18,19] Thus, ICZ dissolution was investigated in FaSSIF with USP apparatus II and GIS, and those results were compared with the results in $SIF_{pH6.5}$.

In the assessment of oral dosage forms, the intestinal permeability for drug substance has to be evaluated because solubility-enhancing technologies often negatively affect the permeation rate of the drug.[20,21] Therefore, in this study, permeation potential of 2 dosage forms for ICZ was assessed in human colonic carcinoma cell line (Caco-2 cell) monolayer system, which is a golden standard for *in vitro* permeation study.[22] To our best knowledge, this Caco-2 permeability study will be the first attempt to compare the permeation potential of 2 commercially available oral dosage forms of ICZ.

The objectives of this present study were to (1) predict *in vivo* drug dissolution profiles of 2 dosage forms of ICZ with GIS and USP II; (2) investigate the suitability of FaSSIF to predict better *in vivo* in GIS; (3) assess whether the different drug concentration levels observed in GIS by different formulation technology will enhance the oral drug absorption by Caco-2 monoepithelial transport studies.

Materials and Methods

Chemicals

ICZ 100-mg capsules (SPORANOX® 100-mg capsules; Janssen Pharmaceutical USA, Titusville, NJ) and ICZ 10 mg/mL oral solution (SPORANOX® 10-mg/mL oral solution, Janssen Pharmaceutical USA) were obtained through University of Michigan Hospital. ICZ, potassium chloride, potassium phosphate monobasic, sodium chloride, and Lucifer yellow CH dipotassium salt were purchased from Sigma-Aldrich Chemicals Corporation (St. Louis, MO). Acetonitrile, trifluoroacetic acid (TFA), and methanol were purchased from Fisher Scientific Inc. (Pittsburgh, PA) and used as received. All chemicals were either analytical or HPLC grade. For Caco-2 experiment, all cell culture reagents were obtained from Life Technologies (Grand Island, NY).

Biorelevant Media

FaSSIF was prepared by dissolving simulated intestinal fluid (SIF) powder according to manufacturer's instruction (Biorelevant.com, Croydon, Surrey, UK). FaSSIF contains 3-mM sodium taurocholate (NaTC) and 0.75-mM lecithin in 28.7-mM potassium phosphate buffer with 103.4-mM potassium chloride at pH 6.5, which composition is derived from Galia et al.[19]

Dissolution Study With USP Apparatus II

The dissolution studies of ICZ capsule and oral solution were performed with a Hanson SR6 Dissolution Test Station (Chatsworth, CA). Either a 100-mg capsule or 10-mL oral solution of ICZ was dosed in 300 mL of the dissolution media, which is either a SIF ($SIF_{pH6.5}$, 50-mM sodium phosphate buffer with 15.4-mM sodium chloride at pH 6.5) or FaSSIF. Dissolution studies were conducted at a rotational speed of 50 rpm at 37°C. The volume (300 mL) of dissolution media was used to compare the results in the other *in vitro* dissolution apparatus (GIS) and to predict better *in vivo*. Samples (200 μL) were manually obtained at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 45, 60, 90, 120, 150, and 180 min. All samples were immediately centrifuged at 9000*g* for 1 min to yield supernatant. The supernatants (100 μL) were collected and mixed with the equal volume of methanol. Dissolved drug concentration was measured by HPLC analysis.

Dissolution Study With GIS

In vitro dissolutions of 2 dosage forms of ICZ were assessed with GIS following previously described method.[6,9] The GIS dissolution condition represents more physiological condition of human GI tract in fasted state than USP apparatus II. The diagram of the GIS is shown in Figure 1. The GIS has 3 chambers, representing the stomach, the duodenum, and the proximal jejunum. Initially, the gastric chamber ($GIS_{stomach}$) has 50 mL of simulated gastric fluid (SGF) at pH 2.0 ($SGF_{pH2.0}$, 10^{-2} N HCl with 34.2-mM sodium chloride) with 250 mL of distilled water as the dose volume. The duodenal chamber ($GIS_{duodenum}$) is filled with 50 mL of either $SIF_{pH6.5}$ or FaSSIF, and the jejunal chamber ($GIS_{jejunum}$) is empty at first.

To start the experiment, either a 100-mg capsule or 10-mL oral solution of ICZ was dosed into the $GIS_{stomach}$. As for oral solution, 240-mL dose volume instead of 250 mL was also adopted for the comparison purpose (in total 300 mL). ICZ oral solution was dosed and mixed in the stomach for 1 min before starting the dissolution study to disperse the drug. At time 0, the gastric components were pumped into the $GIS_{duodenum}$ through a connected tube. The fluid transfer rate from the $GIS_{stomach}$ to the $GIS_{duodenum}$ was controlled by computer to decrease the gastric fluid volume at first-order rate, which was set at 8 min as a gastric half-emptying time. The fluid volume in the $GIS_{stomach}$ at time t ($V_{stomach}$) can be represented as follows: