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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:

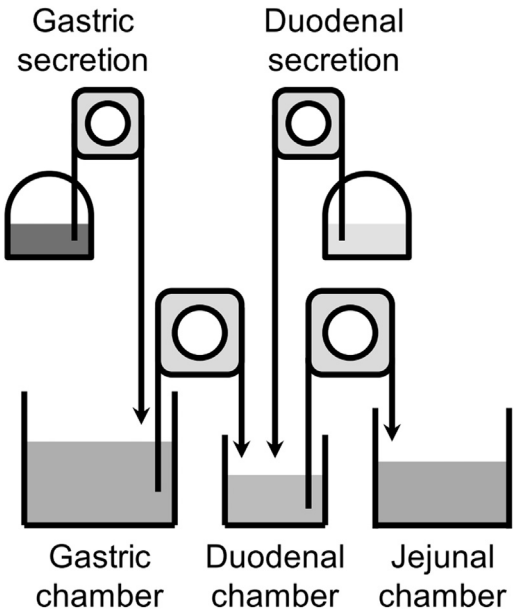

Figure 1. The diagram of GIS.

$$V_{stomach} = V_{stomach,initial} \times \exp(-\ln(2) \times t/8) \quad (1)$$

where $V_{stomach,initial}$ is the initial fluid volume in the $GIS_{stomach}$ which is in total 300 mL (50 mL of SGF with 250 mL of dose volume for a capsule or 50 mL of SGF with 240 mL of dose volume for oral solution). The duodenal components were also pumped into the $GIS_{jejunum}$ at an appropriate rate to maintain the fluid volume in the $GIS_{duodenum}$ at constant (50 mL). To mimic the gastric and duodenal secretions, the gastric secretion fluid ($SGF_{pH2.0}$) and the duodenal secretion fluid were introduced into the $GIS_{stomach}$ and $GIS_{duodenum}$ at the constant rate of 1 mL/min, respectively. All fluid transfers were conducted by peristaltic pumps (Ismatec® REGLO pump; IDEX Health and Science, Glattbrugg, Switzerland).

When $SIF_{pH6.5}$ was used as a duodenal fluid in the GIS, 100-mM phosphate buffer at pH 6.5 was adopted as a duodenal secretion fluid to maintain the phosphate concentrations in the $GIS_{duodenum}$ and $GIS_{jejunum}$. Meanwhile, in condition with FaSSIF as a duodenal fluid, 2 × concentrated FaSSIF (2 × FaSSIF) and 4 × concentrated FaSSIF (4 × FaSSIF) were examined as a duodenal secretion fluid to maintain the pH and bile salt concentration in a physiologically relevant range. All experiments were performed at 37°C. The paddle speeds in the $GIS_{stomach}$ and $GIS_{duodenum}$ were controlled to insure adequate mixing.[9] The $GIS_{jejunum}$ was stirred at a constant speed using a stir bar. All transfer and secretion pumps were stopped at 45 min, but dissolution studies were conducted until 180 min. Samples (200 μL) were manually obtained at 0, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 60, 90, 120, 150, and 180 min. All samples were immediately centrifuged at 9,000×*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.

Comparison of In Vitro *Dissolution Results to Clinical Results*

To determine the suitability of these *in vitro* dissolution apparatuses to predict *in vivo* dissolution by evaluating 2 dosage forms of ICZ, obtained drug concentration-time profile in each apparatus was converted to drug amount in solution-time profile and was compared with ICZ concentration in plasma-time profile in human.[23,24] There is no crossover trial to directly compare the pharmacokinetics of those 2 different oral formulations in fasted state. Therefore, pharmacokinetic parameters were obtained from 2 separate clinical studies (Table 1).

Table 1
Pharmacokinetic Parameters of Itraconazole 2 Dosage Forms in Japanese Healthy Subjects After an Administration of Single Dose (100 mg) in Fasted State

Formulation	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC (ng × h/mL)
Capsule[a]	53.2 ± 24.5	3.6 ± 0.9	32.8 ± 7.8	1326 ± 573[b]
Oral solution[c]	309.9 ± 43.8	1.8 ± 0.4	24.1 ± 9.6	2843 ± 703[d]

[a] Reference: Oguchi et al.[24]
[b] $AUC_{(0\text{-}23hr)}$.
[c] Reference: Tei et al.[23]
[d] $AUC_{(0\text{-}\infty)}$.

From *in vitro* drug amount in solution-time profile, area under the drug amount in solution-time curve (AUDC) from 0 min to 180 min was calculated for each dosage form in USP apparatus II and in GIS and the ratios of $AUDC_{oral\ solution}$ to $AUDC_{capsule}$ were calculated based on their dissolution results using following Equations 2 and 3.

$$\text{AUDC ratio in USP} = \frac{AUDC_{oral\ solution}\ \text{in USP apparatus II}}{AUDC_{capsule}\ \text{in USP apparatus II}} \quad (2)$$

$$\text{AUDC ratio in GIS} = \frac{AUDC_{oral\ solution}\ \text{in GIS}}{AUDC_{capsule}\ \text{in GIS}} \quad (3)$$

For the calculation of AUDC in GIS, dissolved drug in the duodenal and jejunal chambers is regarded as the bioavailable drug because ICZ has a highly permeable property. The drug absorption from the stomach is assumed to be negligible. These AUDC ratios were compared with area under the plasma drug concentration-time curve (AUC) ratio ($AUC_{oral\ solution}/AUC_{capsule}$) in human clinical studies.

Cell Culture

Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD) and cultured in an atmosphere of 5% CO_2 and 90% relative humidity at 37°C. Cells were routinely maintained in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% fetal bovine serum, 100-U/mL penicillin, 100-μg/mL streptomycin, and 1% nonessential amino acids.

Caco-2 Monolayer Transepithelial Transport Assay

Caco-2 cells between passages 39 and 43 were seeded on collagen-coated polytetrafluoroethylene membrane inserts with 0.4-μm pore size and 12-mm diameter (12-well Transwell; Corning Inc., Corning, NY). Cells were grown for 21-23 days, and medium was changed every 2-3 days. Only monolayers with a transepithelial electrical resistance (TEER) higher than 200 Ω × cm^2 were used for this study. TEER values were measured by using a Millicell-ERS epithelial Voltohmmeter (Millipore Corporation, Bedford, MA).

On the day of experiment, Caco-2 cell monolayers were preincubated with FaSSIF in the apical side and with 1% bovine serum albumin (BSA)-containing transport buffer (5-mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 5-mM D-glucose, 1-mM calcium chloride, 0.5-mM magnesium chloride, 145-mM sodium chloride, 1-mM sodium dihydrogen phosphate, 3-mM potassium chloride at pH 7.4) in the basolateral side for 30 min. At time 0, the

blank buffer in the apical and basolateral sides were replaced by 0.5 mL of ICZ-containing suspension and 1.5 mL of fresh 1% BSA-containing transport buffer, respectively. In the basolateral side, 1% BSA-containing transport buffer was used to maintain the sink condition as described previously.[25] At predefined time points (30, 60, 90, and 120 min), 0.1 mL of samples were taken from the basolateral side and replaced with the same volume of 1% BSA-containing transport buffer. Samples were also taken from the apical side at 0 and 120 min. During the experiment, the plate was kept on an orbital shaker at 50 rpm in 37°C incubator. Apical samples were immediately centrifuged at 9000*g* for 1 min, and supernatants were mixed with the equal volume of methanol for HPLC analysis. Basolateral samples were mixed with twice volume of methanol and vortexed for 1 min. After centrifugation (9000*g* for 3 min), deproteinized supernatants were analyzed with HPLC. The apparent permeability coefficient of ICZ (P_{app} [cm/s]) was determined from the slope of the time course of cumulative drug amount from 90 min to 120 min, assuming that drug concentration in the apical compartment was constant for final 30 min (Eq. 4).

$$P_{app} = \frac{X_{120min,BL} - X_{90min,BL}}{S \times C_{120min,AP}} \times \frac{1}{T} \quad (4)$$

where $X_{90min,BL}$ and $X_{120min,BL}$ is the cumulative amount of ICZ into the basolateral compartment at 90 and 120 min, respectively. S is the growth area of Transwell for Caco-2 cells (1.12 cm^2), and $C_{120min,AP}$ is the concentration of ICZ in the apical compartment at 120 min. T is incubation time (1800 s).

Preparation of ICZ Suspension for Caco-2 Cell Monolayer Transepithelial Transport Assay

Test suspensions containing ICZ in apical buffer media were prepared with ICZ capsule formulation, ICZ oral solution formulation, or ICZ powder. Briefly, 100 mg of ICZ capsule or oral solution was dosed into the $GIS_{stomach}$ with FaSSIF in the $GIS_{duodenum}$. Drug suspensions were collected at the time to maximum concentration (T_{max}) in the $GIS_{jejunum}$, which were 60 min for capsule formulation and 5 min for oral solution formulation. To prepare the suspension from ICZ powder for comparison purposes, 100-mg ICZ was mixed into 300 mL of FaSSIF and stirred by a magnetic stirrer for at least 24 h at room temperature. To start the transepithelial transport study, obtained drug suspensions were applied to the apical compartment.

Evaluation of the Integrity of Caco-2 Cell Monolayer

To insure the monolayer integrity, TEER values were measured before and after the study, and the permeability of lucifer yellow was assessed after ICZ transepithelial transport assay. ICZ drug suspension in apical side was replaced by 0.5-mL transport buffer (pH 6.8) containing 30-μg/mL lucifer yellow to start the evaluation. After 60 min, sample was collected from the basolateral side, and fluorescence in samples was measured at Synergy HT (BioTek, Winooski, VT) with excitation of 400/30 nm and emission of 528/20 nm in a 96-well black plate. The apparent permeability coefficient of lucifer yellow (P_{app} [cm/s]) was calculated using following Equation 5.

$$P_{app} = \frac{V}{S \times C_{0,AP}} \times \frac{C_{T,BL}}{T} \quad (5)$$

where V is the volume in the basolateral compartment (1.5 mL). S is the growth area of Transwell for Caco-2 cells (1.12 cm^2). $C_{0,AP}$ is the initial concentration of lucifer yellow at the apical side (30 μg/mL). T is incubation time (3600 s), and $C_{T,BL}$ is the concentration of lucifer yellow in the basolateral side at time T. Monolayers with lucifer yellow $P_{app} < 1 \times 10^{-6}$ cm/s were considered to have appropriate barrier functions.[26,27]

HPLC Analytical Method

ICZ concentration was measured by gradient HPLC method using a Water HPLC system (Waters Inc., Milford, MA). The HPLC system was composed of 2 Waters pumps (model 515), a Waters autosampler (WISP model 712), and a Water UV detector (996 photodiode array detector) controlled by Waters Millennium 32 software (version 3.0.1). A ZORBAX Eclipse XDB-C18 column (3.5 μm, 4.6 × 150 mm) equipped with a guard column was used for the separation. The mobile phases were 0.1% TFA containing water (solvent A) and 0.1% TFA containing acetonitrile (solvent B). The flow rate was set to 1.0 mL/min at room temperature, and solvent B gradient was changing from 20% to 65% at a rate of 11.5% per minute during a 12-min run. To determine drug concentration, 100 μL of sample was injected into HPLC. The wavelength of the UV detector was set at 263 nm.

Statistical Analysis

All dissolution studies including Caco-2 experiments were performed in triplicate or quadruplicate. All results were expressed as mean ± SD.

Results

In Vitro *Dissolution Profiles of ICZ 2 Different Oral Dosage Forms in USP Apparatus II*

Either an ICZ 100-mg capsule or ICZ 10-mL oral solution (100 mg) was tested in USP apparatus II with 300 mL of either $SIF_{pH6.5}$ or FaSSIF. Drug concentration-time profile for each dosage form is shown in Figure 2. In $SIF_{pH6.5}$, drug dissolution of ICZ capsule reached a plateau at 90 min, and drug concentration was approximately 1 μg/mL, whereas ICZ oral solution was once dosed in $SIF_{pH6.5}$, drug concentration instantly went down to 50 μg/mL. This rapid reduction would be caused by immediate disassociation of ICZ from HP-β-CD, but that concentration was maintained for 180 min (Fig. 2a).

Drug concentration-time profiles in FaSSIF are presented in Figure 2b. Drug dissolution of ICZ capsule in FaSSIF was dramatically increased up to 9.5 ± 0.4 μg/mL, which was 10-fold higher than in $SIF_{pH6.5}$. As for oral solution, drug concentration went down to 106.5 ± 4.9 μg/mL immediately after dosing and was gradually decreased to 57.6 ± 8.5 μg/mL at 180 min.

Buffer Adjustment of FaSSIF for GIS

When $SIF_{pH6.5}$ was used as the duodenal fluid of GIS in previous studies, the pH levels in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were in a physiologically relevant range. However, FaSSIF (28.7-mM phosphate buffer) has weaker buffer capacity than $SIF_{pH6.5}$ (50-mM phosphate buffer), thus phosphate buffer concentration in the duodenal secretion fluid shall be adjusted considering pH-time profiles in the $GIS_{duodenum}$ and $GIS_{jejunum}$. In addition, bile salts concentration of FaSSIF should also be taken into account. To establish an optimal experimental condition, pH-time profiles in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were measured without drug and were shown in Figure 3. When $SIF_{pH6.5}$ and 2 × concentrated $SIF_{pH6.5}$ (100-mM phosphate buffer) were used as the initial duodenal and the duodenal secretion fluids, the pH in the $GIS_{duodenum}$ fluctuated from 6.5 to 5.9 ± 0.1 at 9 min because of the acid influx and went back to 6.3 ± 0.1 at 45 min. The pH in the $GIS_{jejunum}$ was stable and stayed between 6.3 and 6.5. On the other hand, in condition with

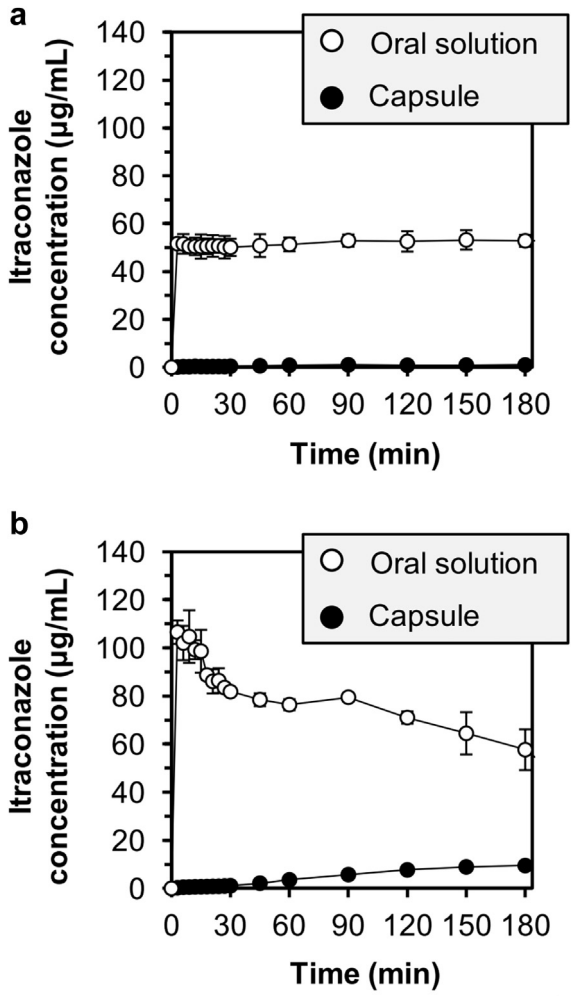

Figure 2. Dissolved drug concentration-time profiles of itraconazole in USP apparatus II with $SIF_{pH6.5}$ (a) or with FaSSIF (b). A itraconazole (SPORANOX®) 100-mg capsule (black circles) and 10 mL of itraconazole (SPORANOX®) 10 mg/mL oral solution (white circles) were dosed into 300 mL of either $SIF_{pH6.5}$ or FaSSIF, and dissolved drug concentration was determined up to 180 min. Each data point represents mean ± SD (n = 3).

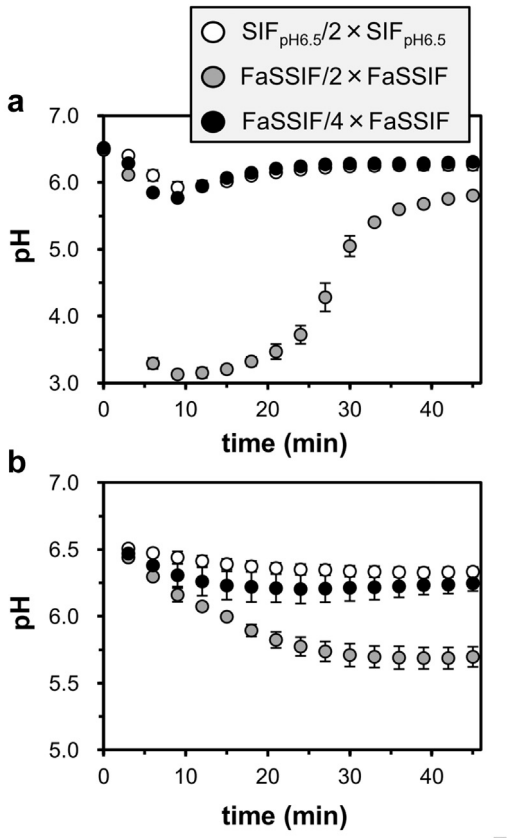

Figure 3. pH-time profiles in the duodenal (a) and the jejunal chambers (b) with different buffer conditions in GIS. White circles represent the condition with 50-mM phosphate buffer ($SIF_{pH6.5}$) as an initial duodenal fluid and 100-mM phosphate buffer as a duodenal secretion fluid. Gray circles indicate the condition with FaSSIF and 2 × concentrated FaSSIF as a duodenal fluid and a duodenal secretion fluid, respectively. Black circles represent the condition with FaSSIF as a duodenal fluid and 4 × concentrated FaSSIF as a duodenal secretion fluid. Each data point represents mean ± SD (n = 3).

FaSSIF as the initial duodenal fluid and with 2 × FaSSIF as the duodenal secretion fluid, the duodenal pH was dropped to 3.1 at 9 min. When 4 × FaSSIF was adopted as the duodenal secretion fluid, the pH-time profiles in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were similar with those in condition with $SIF_{pH6.5}$.

The bile salt concentration-time profiles were calculated based on the fluid influx/efflux rate and bile salt concentration in the fluids. Figure 4 represents NaTC concentration-time profile in the $GIS_{duodenum}$ and $GIS_{jejunum}$. NaTC concentration in the $GIS_{duodenum}$ was remarkably decreased at the initial phase (~10 min) because of the rapid influx of the gastric fluid, which does not contain NaTC. When 4 × FaSSIF was used as the secretion fluid, NaTC concentrations in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were gradually increased from 0.7 and 1.1 mM up to 3.2 mM and 1.4 mM at 45 min, respectively. On the other hand, 2 × FaSSIF could not recover NaTC concentrations in the $GIS_{duodenum}$ and $GIS_{jejunum}$. Considering these results, 4 × FaSSIF was adopted as secretion media and dissolution studies were conducted as follows.

In Vitro *Dissolution Profiles of 2 Different Oral Dosage Forms for ICZ in GIS With* $SIF_{pH6.5}$

In vitro dissolution of 2 oral dosage forms for ICZ was assessed in GIS with $SIF_{pH6.5}$. The drug concentration-time profiles are shown in Figure 5. When an ICZ 100-mg capsule was tested in GIS, drug concentration in the $GIS_{stomach}$ was reached to 32.9 ± 3.5 μg/mL. However, such a level of drug concentration was not observed in the subsequent duodenal and jejunal chambers. The maximum drug concentrations in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were 2.0 ± 0.6 μg/mL and 0.7 ± 0.1 μg/mL, respectively. When 10 mL of ICZ 10-mg/mL oral solution (100 mg) was dosed, the initial drug concentration in the $GIS_{stomach}$ was 199.9 ± 8.7 μg/mL, and it was decreased to 138.0 ± 12.7 μg/mL at 44 min because the dilution by the introduction of gastric secretion. This higher drug concentration triggered high drug concentration in the $GIS_{duodenum}$ and $GIS_{jejunum}$. The maximum drug concentrations in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were 50.4 ± 4.3 μg/mL at 11 min and 31.2 ± 1.4 μg/mL at 20 min, respectively. Drug concentrations in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were not maintained throughout the dissolution study (180 min) and went down to 5.0 ± 0.5 μg/mL and 2.8 ± 0.3 μg/mL at 180 min in the $GIS_{duodenum}$ and $GIS_{jejunum}$, respectively.

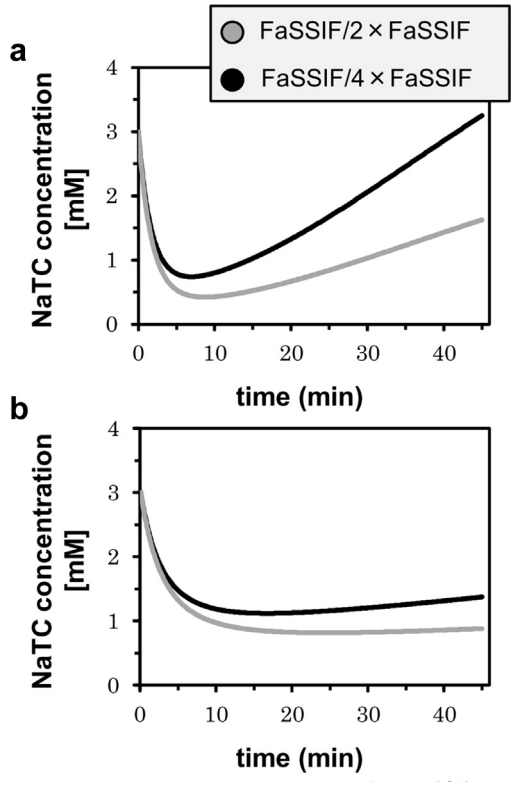

Figure 4. Simulated NaTC concentration-time profiles in the duodenal (a) and the jejunal chambers of GIS (b). Gray line represents the condition with FaSSIF as a duodenal fluid and 2 × concentrated FaSSIF as a duodenal secretion fluid. Black line indicates the condition with FaSSIF as a duodenal fluid and 4 × FaSSIF as a duodenal secretion fluid.

In Vitro *Dissolution Profiles of 2 Different Oral Dosage for ICZ Forms in GIS With FaSSIF*

The concentration-time profiles of 2 dosage forms for ICZ were also demonstrated in GIS with FaSSIF, and the results are shown in Figure 6. An ICZ 100-mg capsule showed the same drug concentration-time profile in the $GIS_{stomach}$ as with $SIF_{pH6.5}$ but exhibited higher drug concentration in the $GIS_{duodenum}$ and $GIS_{jejunum}$ because of the solubilization effects by bile salts in FaSSIF. The observed maximum drug concentrations were 14.3 ± 0.3 μg/mL (60 min) and 7.6 ± 0.7 μg/mL (60 min) in the $GIS_{duodenum}$ and $GIS_{jejunum}$, which were 7-fold and 11-fold higher concentrations than those in GIS with $SIF_{pH6.5}$, respectively. When 10 mL of ICZ 10 mg/mL oral solution (100 mg) was assessed in GIS with FaSSIF, the maximum drug concentrations were 54.3 ± 7.2 μg/mL (2 min) and 40.6 ± 6.2 μg/mL (5 min) in the $GIS_{duodenum}$ and $GIS_{jejunum}$, respectively. These values were significantly higher than those of ICZ capsule in GIS with FaSSIF until 60 min. However, these concentrations were gradually decreased and at the end of the study (180 min), drug concentrations in the $GIS_{duodenum}$ and $GIS_{jejunum}$ went down to 3.6 ± 0.4 μg/mL and 3.7 ± 0.5 μg/mL, respectively.

Drug Amount in Solution-Time Profiles in GIS

Drug amount in solution for each dosage form of ICZ was calculated at each time point in the GIS. Figure 7 presents the sum of drug amount in solution in the $GIS_{duodenum}$ and $GIS_{jejunum}$. In $SIF_{pH6.5}$, drug amount in solution for a capsule formulation was 0.4 ± 0.02 mg as the highest value at 38 min. Drug amount in solution for oral solution in GIS with $SIF_{pH6.5}$ reached up to 12.6 ± 1.0 mg at 41 min and then gradually reduced to 1.3 ± 0.1 mg at 180 min. In condition with FaSSIF, ICZ capsule exhibited relatively high drug amount in solution in the intestinal chambers (3.6 ± 0.3 mg at 60 min), and the amount in solution was maintained during the study (up to 180 min). As for oral solution, the maximum drug amount in solution in the intestinal chambers (12.8 ± 1.6 mg at 38 min) was almost the same as that in $SIF_{pH6.5}$ (12.6 ± 1.0 mg at 41 min), but drug amount in solution in FaSSIF was dramatically dropped at 60 min, whereas one in $SIF_{pH6.5}$ was maintained up to 90 min, which led different drug amount in solution-time profiles.

The Comparison of In Vitro *Dissolution With Clinical Pharmacokinetic Parameters*

In vitro dissolution studies in USP apparatus II and GIS were summarized in parallel with human clinical data after a single dose of ICZ 100 mg in fasted state (Table 2). AUDC ratios were calculated for both USP apparatus II and GIS with $SIF_{pH6.5}$ or FaSSIF following Equations 2 and 3. AUDC ratio in USP was 66.0 (in $SIF_{pH6.5}$) and 14.2 (in FaSSIF), respectively, and there are huge discrepancy in those ratios between *in vitro* dissolution results and *in vivo* study results (2.1). AUDC ratio in GIS with $SIF_{pH6.5}$ was 31.4, which is far from human AUC ratio. Meanwhile, AUDC ratio in GIS with FaSSIF was 1.8, which is significantly lower than other AUDC ratios and is close to the ratio from the clinical study.

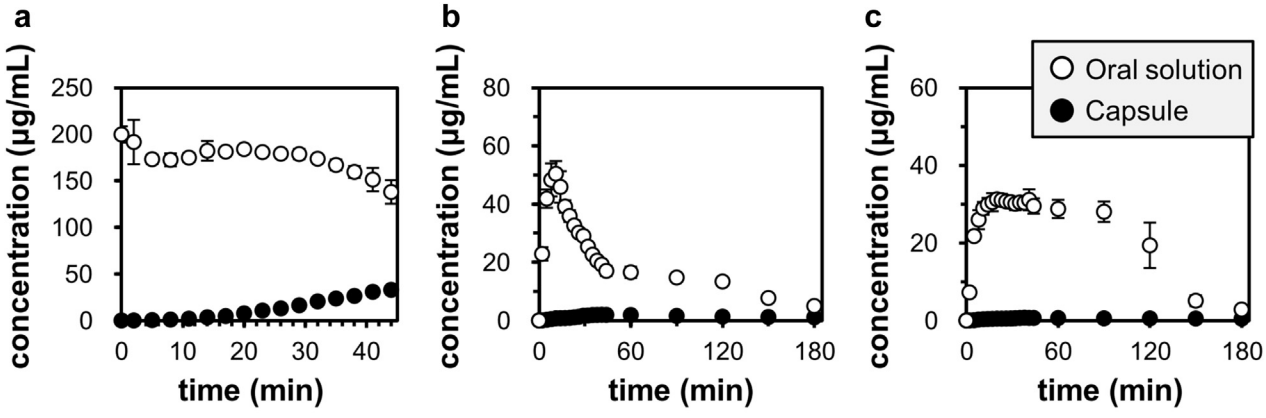

Figure 5. Drug concentration-time profiles of itraconazole capsule and oral solution in the gastric (a), duodenal (b), and jejunal chambers (c) in GIS with 50-mM phosphate buffer ($SIF_{pH6.5}$). Black and white circles represent dissolution profiles of itraconazole capsule and oral solution, respectively. Each data point represents mean ± SD (n = 3).

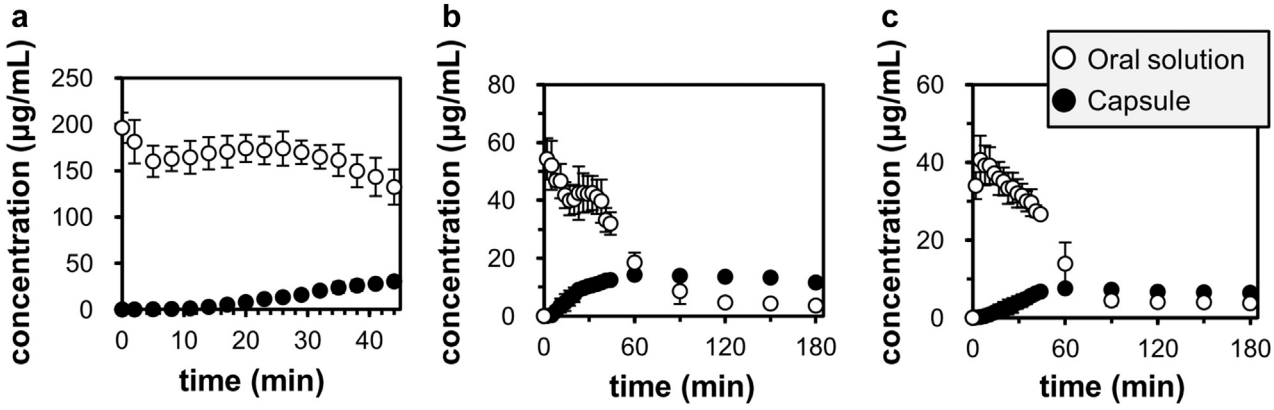

Figure 6. Drug concentration-time profiles of itraconazole capsule and oral solution in the gastric (a), duodenal (b), and jejunal chambers (c) in GIS with FaSSIF. Black and white circles represent dissolution profiles of itraconazole capsule and oral solution, respectively. Each data point represents mean ± SD (n = 3).

Permeation Study via Caco-2 Cell Monolayer

ICZ suspension obtained from the jejunal chamber of the GIS with FaSSIF was applied to the apical side of Caco-2 cell monolayer. The suspension in the $GIS_{jejunum}$ 60 min after ICZ capsule dosing or 5 min after ICZ oral solution dosing was added to the apical side to start the transepithelial study with Caco-2 monolayer. At each time point, each dosage form exhibited maximum drug concentration in the $GIS_{jejunum}$ (Fig. 6c). For comparison purposes, ICZ pure powder was saturated in FaSSIF and used for the study. The equilibrium of ICZ solubility in FaSSIF was achieved within 4 h, and solubility was maintained up to 28 h (data not shown), thus ICZ suspension with stirring for 24 h was provided for the study.

To check the compatibility of Caco-2 cell monolayer with ICZ suspension, TEER values were measured before (0 min) and after (120 min) transport study. As shown in Table 3, no significant attenuation in TEER values was observed. Moreover, the P_{app} of lucifer yellow, which was measured after transepithelial assay of ICZ formulations, was less than 1×10^{-6} cm/s in all tested groups, suggesting that the integrity of Caco-2 cell monolayer was maintained during the study.

Cumulative drug amount in the basolateral compartment was plotted against time up to 120 min (Fig. 8). When ICZ pure powder in FaSSIF was added to the apical compartment, no permeated drug was detected (<0.03 μg). This might be the result of its poor aqueous solubility and the low drug concentration applied in the apical side. Whereas ICZ from an oral capsule formulation, generated in the jejunal chamber of GIS with FaSSIF, detectably appeared in the basolateral side and the drug amount in the basolateral side after 120 min was 0.21 ± 0.00 μg. ICZ permeation from oral solution in the $GIS_{jejunum}$ with FaSSIF was enhanced up to 0.43 ± 0.04 μg at 120 min.

The concentration of ICZ in the apical compartment before and after transepithelial study was shown in Table 4. As for ICZ suspensions from pure powder in FaSSIF and capsule in the $GIS_{jejunum}$ with FaSSIF, initial drug concentrations in the apical compartment were maintained during the study. In contrast, ICZ suspension from oral solution in the $GIS_{jejunum}$ exhibited significant reduction of drug concentration in the apical side (from 41.5 ± 2.2 μg/mL at 0 min to 22.9 ± 0.8 μg/mL at 120 min) because of precipitation during the study. Apparent permeability of ICZ was calculated based on Equation 4. As shown in Table 4, no meaningful permeation from ICZ pure powder was observed, thus permeability was not calculated for ICZ pure powder. On the other hand, both ICZ oral formulations exhibited similar permeability ($3.29 \pm 0.85 \times 10^{-6}$ cm/s for ICZ capsule, $2.50 \pm 0.87 \times 10^{-6}$ cm/s for ICZ oral solution).

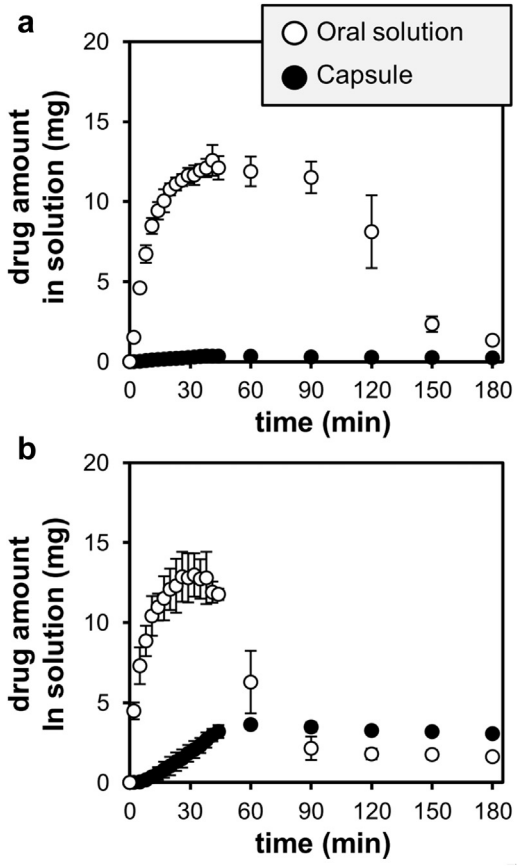

Figure 7. Drug amount in solution-time profiles of itraconazole capsule and oral solution in the sum of duodenal and jejunal chambers in GIS with 50-mM phosphate buffer ($SIF_{pH6.5}$) (a) or with FaSSIF (b). Black and white circles represent dissolution profiles of itraconazole capsule and oral solution, respectively. Each data point represents mean ± SD (n = 3).

Discussion

In terms of formulation strategy, ICZ is one of the most widely investigated pharmaceutical drugs.[28-34] It is due to ICZ's poor aqueous solubility and low wettability. Many attempts have been carried out to improve the solubility and oral absorption of ICZ so far. Some formulation designs succeeded in enhancing *in vitro*

Table 2
The Comparison of *In Vitro* Dissolutions With *In Vivo* Pharmacokinetics of Itraconazole 2 Dosage Forms

In Vitro Device/Clinical PK Data	AUDC Ratio, AUC Ratio (Oral Solution/Capsule)
USP apparatus II	
$SIF_{pH6.5}$	66.0
FaSSIF	14.2
GIS	
$SIF_{pH6.5}$	31.4
FaSSIF	1.8
Clinical data	2.1

PK, pharmacokinetic.

dissolution and in achieving higher systemic exposure in preclinical animals or human.[28-30] However, other technologies failed to enhance the oral bioavailability of ICZ even though they successfully exhibited higher dissolution profile in *in vitro* dissolution studies.[31-34] In those failed studies, *in vitro* dissolution tests, which aimed to distinguish the potency in achieving high drug exposure among several formulations, were demonstrated only in a vessel with fixed pH and constant fluid volume like USP apparatus I and II. This fact strongly suggests that such a simple dissolution test does not represent the complexity of human GI tract, and thus, it is not suitable to evaluate ICZ formulations. In human GI tract, orally administered drug will be exposed to an unstable environment, which is typified by a drastic pH increase from the stomach to the proximal small intestine. Because ICZ is classified into BCS class IIb, significant pH change in the GI tract may deeply affects its dissolution profile. According to BCS subclassification approach, BCS class IIb drugs should be assessed in multicompartmental dissolution apparatus which incorporates physiological changes in the GI tract.[35] Thus, GIS may be an appropriate tool to evaluate oral formulations of ICZ.

Here, the dissolution profiles of commercially available oral dosage forms for ICZ, a capsule and an oral solution, were assessed in USP apparatus II and GIS to investigate the *in vivo* predictability with buffer media of $SIF_{pH6.5}$ and FaSSIF. A capsule formulation is the first marketed oral dosage form and is containing ICZ as a solid dispersion with hydroxypropyl methyl cellulose on an inert sugar sphere.[17,36] The oral bioavailability of ICZ capsule is moderate (55%) in fed state but is pretty low in fasted state.[11] Therefore, ICZ capsule is recommended to be taken with food, but some patients are intolerant of taking solid food and swallowing the capsules, resulting in unsuccessful treatment. Oral solution formulation was secondly developed to overcome the limitations of the capsules. In an oral solution, ICZ is solubilized by HP-β-CD at a concentration of 40% with 10% propylene glycol as a cosolvent.[14-16] HP-β-CD is a derivative of cyclodextrin and is capable of forming an inclusion complex containing lipophilic molecules like ICZ. This formulation improves the oral bioavailability even in fasted state by enhancing the solubility as well as the stability of ICZ (Table 1).

To test the physiological relevance of *in vitro* dissolution tests, biorelevant dissolution media, FaSSIF, were used and compared

Table 3
Caco-2 Cell Monolayer Integrity During Transport Study of Itraconazole Suspension, Confirmed by TEER Value and Apparent Permeability of Lucifer Yellow (n = 3-4, Mean ± SD)

Itraconazole Suspension	Apical Media	TEER (Ω × cm^2)		Lucifer Yellow $P_{app} \times 10^{-6}$ [cm/s]
		0 min	120 min	
Pure powder	FaSSIF	300 ± 34	279 ± 24	0.78 ± 0.11
Capsule	GIS/FaSSIF	317 ± 14	285 ± 24	0.76 ± 0.11
Oral solution	GIS/FaSSIF	311 ± 13	281 ± 26	0.66 ± 0.17

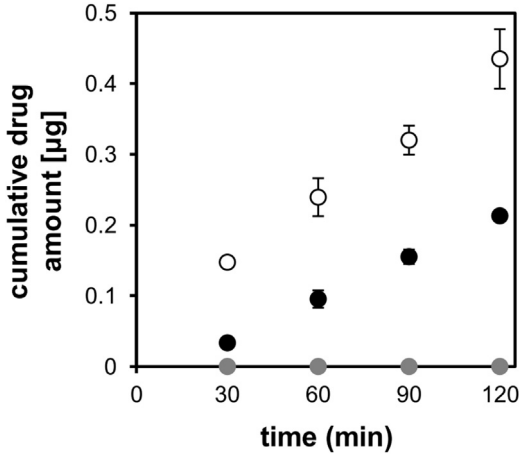

Figure 8. Cumulative amount of itraconazole in the basolateral side of Caco-2 cell monolayer after addition of itraconazole suspensions to the apical compartment; a suspension of itraconazole capsule in the jejunal chamber of GIS with FaSSIF (black circles), oral solution in the jejunal chamber of GIS with FaSSIF (white circles), and itraconazole pure powder in FaSSIF (gray circles), respectively. Each data point represents mean ± SD (n = 3-4).

with phosphate buffer in this study. It is known that there is a log-log correlation between the partition coefficient and solubilization capacity of the bile salts.[37] In fact, saturated concentration of ICZ was <0.01 μg/mL in $SIF_{pH6.5}$ and 0.2 ± 0.0 μg/mL in FaSSIF, indicating significant solubilization effect of bile salts on ICZ. Such an effect was also observed when the dissolution of ICZ capsule was assessed in USP apparatus II (Fig. 2). The concentration-time profile of ICZ capsule was dramatically improved (>10-fold) by FaSSIF in USP apparatus II. Meanwhile, the solubilization effect on drug dissolution-time profile of oral solution was small (<2.1-fold) and was not maintained until 180 min. ICZ in oral solution is already solubilized by HP-β-CD, thus further improvement in drug dissolution was not observed. In both buffers, these 2 dosage forms exhibited higher drug concentration than its saturated concentration (Figs. 5 and 6). These higher drug concentrations come from their formulation effects, suggesting that these 2 formulations will enhance their oral bioavailability, whereas pure ICZ powder itself is not bioavailable.[15] To investigate the predictability of *in vivo* dissolution for ICZ formulations by USP apparatus II, AUDC ratios in USP were calculated based on Equation 2. The ratio of $AUDC_{oral\ solution}$ to $AUDC_{capsule}$ in USP with $SIF_{pH6.5}$ (AUDC ratio in USP with $SIF_{pH6.5}$) was 66.0. This value was much higher than clinical AUC ratio, which is 2.1. In FaSSIF, AUDC ratio in USP with FaSSIF was reduced to 14.2 because of the improved dissolution of a capsule formulation in FaSSIF, but there is still disagreement between *in vivo* AUC ratio and *in vitro* AUDC ratio in USP (Table 2). These results, as well as previous studies which failed to forecast *in vivo* performance of drug products, imply the necessity of the

Table 4
Dissolved Concentration of Itraconazole in the Apical Compartment Before (0 min) and After (120 min) Transport Study, and Apparent Permeability of Itraconazole (n = 3-4, Mean ± SD)

Itraconazole Suspension	Apical Media	Drug Concentration (μg/mL)		Itraconazole $P_{app} \times 10^{-6}$ (cm/s)
		0 min	120 min	
Pure powder	FaSSIF	0.2 ± 0.0	0.2 ± 0.0	NC
Capsule	GIS/FaSSIF	9.0 ± 0.4	8.9 ± 0.7	3.29 ± 0.85
Oral solution	GIS/FaSSIF	41.5 ± 2.2	22.9 ± 0.8	2.50 ± 0.87

NC, not calculated.

development for *in vivo* predictive dissolution methodologies to assess oral dosage forms of ICZ.[31-34]

The experimental condition of GIS with FaSSIF as an intestinal fluid was investigated considering its buffer capacity and bile salts concentration of a duodenal secretion fluid. Because of weaker buffer capacity in FaSSIF compared to $SIF_{pH6.5}$, pH values in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were remarkably dropped when 2 × FaSSIF was used as a secretion fluid (Fig. 3). The lowest pH values were 3.1 ± 0.1 and 5.7 ± 0.1 in the $GIS_{duodenum}$ and $GIS_{jejunum}$, respectively. These values were far from physiological pH levels in human duodenum and proximal jejunum.[38,39] On the other hand, 4 × FaSSIF was able to maintain pH levels in both chambers, and these pH levels matched with observed pH in human. Moreover, NaTC concentration-time curve indicates that 4 × FaSSIF kept NaTC concentration in $GIS_{duodenum}$ in the physiological range and, hence, would be better representation of *in vivo* for the dissolution study with GIS (Fig. 4). In this condition, calculated NaTC concentration in the $GIS_{jejunum}$ was also in physiologically relevant range.[40] Therefore, 4 × FaSSIF was used as a duodenal secretion buffer of GIS. This experimental condition in GIS with FaSSIF may provide more reliable information on *in vivo* dissolution of practically insoluble drugs with low wettability.

In vitro dissolutions of ICZ capsule and oral solution were evaluated in GIS with either $SIF_{pH6.5}$ or FaSSIF. Figure 5 represents the drug concentration-time curves of ICZ capsule and oral solution in GIS with $SIF_{pH6.5}$. Drug dissolution of ICZ capsule reached >30 μg/mL in the $GIS_{stomach}$ at 44 min, which led slightly higher concentration (~2 μg/mL) in the subsequent duodenal chamber than the maximum concentration observed in USP apparatus II (~1 μg/mL). Drug concentration in the $GIS_{jejunum}$ was <0.70 μg/mL. Also in GIS with FaSSIF, ICZ capsule exhibited low dissolution profiles in the $GIS_{duodenum}$ (~15 μg/mL) and $GIS_{jejunum}$ (<8 μg/mL), not extensively higher than that in USP apparatus II (<10 μg/mL; Fig. 6). These results suggest that ICZ capsule formulation does not exhibit high extent of supersaturation in the GIS as observed in case of dasatinib and dipyridamole.[8,9] The possible reason is the inability of ICZ capsule to maintain extensive supersaturation in neutral pH. Similar findings were reported by DiNunzio et al. and Mellaerts et al., illustrating rapid precipitation of ICZ (SPORANOX®) capsule triggered by pH transition from acidic to neutral in the GI tract.[41,42]

In contrast to ICZ capsule, an oral solution exhibited much higher concentration in each chamber of GIS with $SIF_{pH6.5}$ (Fig. 5). When ICZ oral solution was dosed into the $GIS_{stomach}$, ICZ oral solution was immediately dispersed and exhibited high drug concentration (~200 μg/mL) because of the high solubility of ICZ in acidic pH and the solubilization by HP-β-CD. However, drug concentrations in the subsequent chambers were immediately reduced at the same level as observed in USP apparatus II with $SIF_{pH6.5}$ (30-50 μg/mL). This observation suggests pH change from acidic to neutral pH does not have much impact on the concentration of ICZ oral solution. Clinical drug-drug interaction study elucidated that elevated gastric pH, caused by omeprazole, did not affect the oral bioavailability of ICZ oral solution, supporting the observations of ICZ oral solution in the GIS.[43] Interestingly, drug concentrations of oral solution in the $GIS_{duodenum}$ and $GIS_{jejunum}$ were significantly dropped at 90 min. This phenomenon was not observed until 180 min in USP apparatus II. This result is in agreement with a previous report, in which *in vitro* dissolution of ketoconazole-HP-β-CD complex was assessed in a compendial dissolution apparatus and 2-compartmental transfer system.[44] In that experiment, it was reported that the precipitation of ketoconazole occurred faster in the transfer model than in the compendial apparatus. Thus, it is possible that the metastable condition generated by pH changes from acidic to neutral might affect the stability of ICZ complex with HP-β-CD.

When FaSSIF was used as dissolution media, the maximum drug concentrations of oral solution in the $GIS_{duodenum}$ and $GIS_{jejunum}$ did not differ much from those in GIS with $SIF_{pH6.5}$ (Fig. 6). However, solid particles which can easily precipitate without centrifugation were visualized at 60 min in FaSSIF, and the precipitation appeared faster in FaSSIF than in $SIF_{pH6.5}$ (not observed until 90 min), resulting in faster reduction in drug concentration. In general, as lipophilic drugs exhibit higher solubility in FaSSIF than in $SIF_{pH6.5}$, less supersaturation degree in FaSSIF should offer slower precipitation rate. However, ICZ oral solution showed faster precipitation in FaSSIF. This observation can be explained by the molecular interaction between taurocholic acid and HP-β-CD. Because taurocholic acid is reported to form a complex with HP-β-CD, NaTC-HP-β-CD inclusion complex may be generated in place of ICZ-HP-β-CD complex in FaSSIF.[45,46] In that case, disassociated ICZ from HP-β-CD will easily precipitate because of its unstable energy state in aqueous environment. Therefore, faster precipitation of ICZ was observed in FaSSIF despite the higher equilibrium solubility in the media. This competitive replacement of ICZ by taurocholic acid is likely to occur in human GI tract. However, it should be considered that human intestinal fluid contains different molecular forms of bile acids, and the affinity of each bile acid toward HP-β-CD is reportedly different.[40,45] Therefore, because FaSSIF has only a single type of bile acid (taurocholic acid), it is possible that different bile acids make the difference in the precipitation rate for ICZ oral solution.[19,47] For better *in vivo* prediction, further investigation using human intestinal fluid as dissolution media in the intestinal compartments in GIS would be required.

The AUDC ratios in GIS were calculated based on Equation 3 to compare *in vitro* dissolutions of ICZ 2 different oral dosage forms with *in vivo* pharmacokinetic profiles. The AUDC ratio in GIS with $SIF_{pH6.5}$ was 31.4 and higher than clinical AUC ratio (2.1). Meanwhile, the AUDC ratio in GIS with FaSSIF was 1.8, which was close to clinical AUC ratio. Moreover, time to reach the maximum dissolved drug amount (T_{max}) in GIS with FaSSIF was 60 min and 32 min for ICZ capsule and oral solution, respectively. This T_{max} ratio agreed with reported clinical results (Table 1).[23,24] Although the combination of the GIS with *in silico* simulation would be helpful to exactly predict plasma concentration-time profile, the dissolution data obtained in GIS were able to adequately capture the pharmacokinetic profiles of 2 oral dosage forms for ICZ.

In the GIS experiments, 250 mL of water was used as dose volume for the harmonization to the Food and Drug Administration and the European Medicines Agency. On the other hand, the clinical results for ICZ were obtained in Japanese healthy volunteers, who took 150 mL of water as dose volume.[23,24] Even though the difference in the dosing volume, the dose-normalized pharmacokinetics parameters for each formulation in Japanese subjects were similar to those in Americans, indicating that ethnic difference did not affect the pharmacokinetic profiles of ICZ formulations.[11,23,24,48] Therefore, 250 mL of water was used as dose volume in the GIS study to simplify the experimental condition in the GIS and to use this apparatus for general purpose.

The comparison of *in vitro* AUDC with *in vivo* AUC is basically assuming that dissolved ICZ from different dosage forms can be equally absorbed via the intestinal wall and appear in plasma (Table 2). However, solubilizers or cosolvents are known to reduce permeation rate of certain drugs.[20,21] Frank et al. revealed that molecularly dispersed drug can be distinguished from micelle-solubilized drug by inverse dialysis method, and drug in the micelles is not readily available for Caco-2 cell monolayer permeation.[49-51] In our study, not an inverse dialysis but a centrifugation method was used to spin down the precipitated drug in a timely manner. A syringe filtration with a 0.22-μm pore size polyvinylidine difluoride membrane yielded the same drug concentration-time

profile with centrifugation (data not shown). This result represents that the centrifugation can remove microparticles but cannot remove nano-precipitated particles. These facts suggest that measured ICZ solubility in this study should be the sum of molecularly dispersed drug, micelle-incorporated drug, and nano-precipitated drug. To compare *in vitro* dissolution with *in vivo* plasma exposure, truly dissolved drug which can permeate the intestinal wall should be evaluated. Therefore, Caco-2 transepithelial transport assay was combined with the GIS dissolution study to investigate the effect of excipients in drug products on ICZ permeation and to clarify the amount of molecularly dissolved ICZ.

The compatibility of Caco-2 cell monolayer against FaSSIF and/or HP-β-CD is controversial.[20,50,52-55] Thus, Caco-2 cell monolayer integrity was checked by TEER values and lucifer yellow permeability in this study. As shown in Table 3, less than 15% of reduction of TEER and acceptable lucifer yellow permeability ($<1 \times 10^{-6}$ cm/s) indicated that Caco-2 cell monolayer kept its tightness in all tested groups.[26,27] These observations were reasonable because ICZ suspensions obtained from the $GIS_{jejunum}$ contained less NaTC than FaSSIF (Fig. 4b), and excipients in drug products were diluted by $SGF_{pH2.0}$ and FaSSIF to some extent in the GIS.

With functional Caco-2 cell monolayer, ICZ permeability from different dosage forms was assessed. As presented in Figure 8, high concentration of ICZ observed in GIS by oral drug products resulted in enhanced permeation through Caco-2 cell monolayer. Additionally, ICZ capsule and ICZ oral solution exhibited similar permeability (Table 4). These data suggested that these 2 formulations can be equally absorbed, thus dissolution profile of ICZ in the GIS reflects systemic exposure of ICZ.

Although good *in vivo* predictability of ICZ formulations was suggested in the GIS with FaSSIF, there is still a gap between *in vitro* dissolution and human GI tract. In human GI tract, apparent drug concentration in the GI lumen will be reduced by the intestinal absorption.[56,57] On the other hand, the GIS does not incorporate the absorption process, not fully reproduce *in vivo* situation. In case of ICZ formulations, Figure 7 shows that the maximum dissolved drug amount from ICZ oral solution was <13 mg in the GIS (<13% of dosed), suggesting that undissolved ICZ may be still remaining in the GI tract throughout the intestinal transit even though ICZ absorption occurs. Therefore, it can be hypothesized that ICZ absorption from the GI tract is compensated by the dissolution of undissolved ICZ particles to keep the dissolved drug amount at the same level as observed in the dissolution study which has no absorption compartment. Based on this hypothesis, AUDC within the intestinal transit time (180 min) was regarded as bioavailable and compared with plasma AUC.

It also has to be considered that multicompartmental dissolution apparatuses tend to overpredict the precipitation potential of BCS class IIb drugs.[5,58] This is believed that drug absorption will apparently reduce the drug amount in the GI tract, as a result, slowing drug precipitation. Moreover, in assessing HP-β-CD—containing formulations, it should be taken into account that disassociated free drug from HP-β-CD inclusion complex can be absorbed, but HP-β-CD is still remaining in the GI tract because of its inability to be absorbed from the GI tract.[14,16] Therefore, incorporating the absorption process into the GIS dissolution system will advance *in vivo* predictability of the tested drugs.

In this study, *in vitro* dissolution profiles of 2 different oral dosage forms of ICZ (capsule and oral solution) were evaluated in conventional USP apparatus II and in 3-compartmental dissolution apparatus GIS using either $SIF_{pH6.5}$ or FaSSIF as dissolution media. In USP apparatus II, AUDC ratio of ICZ oral solution to capsule was much higher than AUC ratio in human clinical study, indicating the overestimation of ICZ dissolution for oral solution dosage form or the underestimation of ICZ dissolution for oral capsule dosage form. The dissolution studies of 2 dosage forms were also conducted in the GIS using $SIF_{pH6.5}$ as dissolution media in the intestinal chambers, but the results presented discrepancy in the ratios between AUDC and clinical AUC. Meanwhile, the usage of FaSSIF in the GIS significantly improved AUDC ratio to closely match with human AUC ratio. The combination study of Caco-2 cell transepithelial transport study with the GIS revealed that intestinal permeability of ICZ was not different between 2 formulations, suggesting that the observed ICZ concentration in the GIS will directly reflect systemic exposure. Therefore, the GIS dissolution would be a powerful tool to assess the formulations of ICZ and other BCS class II drug formulations. The GIS would be useful for the selection of oral dosage form in drug discovery, formulation development, and Quality by Design initiative.

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