ABSTRACT: The current research evaluated and compared the efficacy of hydroxybutenyl-β-cyclodextrin (HBenBCD) and hydroxypropyl-β-cyclodextrin (HPBCD) as enhancers of itraconazole solubility and oral bioavailability. At 10 wt% cyclodextrin, 17-fold and 3.8-fold increases in itraconazole aqueous solubility were observed in the presence of HBenBCD and HPBCD, respectively. Significant differences in the dissolution of itraconazole in the presence of these two cyclodextrins were also observed. Itraconazole pharmacokinetics is known to exhibit a significant food effect. However, testing in biorelevant media indicated that no food effects should be observed after oral administration of itraconazole:HBenBCD complexes. Formulations of itraconazole with HBenBCD were prepared and these complexes, along with the commercial forms of itraconazole with and without HPBCD (Sporanox®) were administered to male Sprague—Dawley rats by oral and intravenous routes. Intravenous administration of itraconazole formulated with HBenBCD resulted in a higher AUC relative to Sporanox®. When administered as oral solutions, the itraconazole:HBenBCD formulation provided higher oral bioavailability than the Sporanox® oral solution. When administered as solid formulations, the itraconazole:HBenBCD solid formulation provided a 2× increase in oral bioavailability relative to the Sporanox® solid formulation. No food effects were observed with the itraconazole:HBenBCD solid dosage forms. Drug/metabolite ratios were dependent upon the dosage form.

INTRODUCTION

Fungal infection can be a serious medical problem, particularly as a complicating factor in serious illnesses such as hematological malignancy, HIV infection, and chemotherapy-induced neutropenia. A preferred treatment for cutaneous and
systemic fungal infections involves administration of antifungal azole compounds. Antifungal azole compounds are structurally diverse and characterized by having imidazole or triazole functionalities. This class includes drugs such as itraconazole, ketoconazole, fluconazole, saperconazole, miconazole, voriconazole, and several others. These compounds generally have low water solubility, which in most cases translates into low bioavailability. This can have a negative impact on efficacy, side effects, inter- and intra-patient variability, and ultimately the utility of the drug.

Itraconazole (Fig. 1) is a broad-spectrum triazole agent available for the treatment of histoplasmosis, blastomycosis, onychomycosis, and amphotericin B-refractory aspergillosis. Itraconazole is highly effective in vitro against Candida albicans and other Candida species. The effectiveness of itraconazole is in part due to the fact that hydroxyitraconazole, the main metabolite of itraconazole, also has considerable antifungal activity.

Itraconazole is a weakly basic drug (pK₆ ca. 3.7) that was found experimentally to have very poor water-solubility (intrinsic solubility, S₀ = 30 μg/mL). The drug is highly crystalline having a Tₘ of 170°C and a logp-value of 5.66 at pH 8.1. Itraconazole can only be ionized and solubilized in water at very low pH. Itraconazole has limited solubility in organic solvents with CH₂Cl₂ being the preferred solvent. Itraconazole also has limited stability at elevated temperatures and at low pH.

A number of investigators have explored the use of cyclodextrins (CDs) as a means to increase water solubility and bioavailability of antifungal drugs such as itraconazole. CDs are cyclic oligomers of glucose, which typically contain 6, 7, or 8 glucose monomers joined by α,1,4 linkages; these oligomers are commonly called α-CD, β-CD, and γ-CD, respectively. Topologically, CDs form a torus that has a hydrophobic interior and a hydrophilic exterior. This allows the CD to be dissolved in water, where it acts as a host molecule and forms inclusion complexes with hydrophobic guest molecules. This feature has led to the use of CDs in pharmaceutical formulations. Unmodified CDs, particularly β-CD, are relatively crystalline and have limited aqueous solubility. In parenteral formulations, limited solubility is a very serious issue as renal concentration of the unmodified CD can lead to crystallization of the CD and necrotic damage. Fortunately, the solubility of unmodified CDs in water can be significantly increased by the addition of a small number of substituents to the hydroxyl groups of the anhydroglucose monomers.

![Figure 1.](https://example.com/figure1.png) Structures of (a) HBenBCD and (b) itraconazole which were used in this study.

Itraconazole is currently available in multiple formulations under the brand-name Sporanox® (Janssen Pharmaceutica N.V., Titusville, New Jersey). One such formulation is an oral solution (10 mg of itraconazole/mL) containing 400 mg/mL of hydroxypropyl-β-cyclodextrin (HPBCD). Sporanox® oral solution is clear and yellowish in color with a target pH of 2.

Itraconazole is also available as an IV dosage form which is similar to that of the oral liquid form with the notable exception that the target pH is ca. 4.5. Itraconazole is not given as an IV bolus but rather infused over a 1 h period. There is also a hard gelatin capsule form of Sporanox® (100 mg of itraconazole coated on sugar spheres). The solid capsule form does not contain any cyclodextrin. The recommended dose of both the solid formulation and the oral liquid is 200 mg (2 capsules or 20 mL) given once daily.

In humans, the bioavailability of itraconazole from the oral solution has been reported to be 55%, which is 37% higher than that observed from the capsule form. Hence, the solution and capsule dosage forms cannot be used interchangeably. The capsule formulation is supposed to be taken after a full meal, which limits its utility as many patients are unable to take solid food and gastrointestinal events may limit food intake. Higher oral bioavailability with the oral liquid dosage form is observed in humans when the patient has fasted. Pharmacokinetic (PK) studies for itraconazole have been reported in several species including rat, rabbit, and guinea pigs. Results from studies involving Sprague-Dawley rats and in mice indicate that the oral bioavailability of itraconazole was influenced by the dosage form. The oral bioavailability of itraconazole when administered as oral solution and capsule dosage forms was 35% and 10%, respectively. One should also note that itraconazole is both a CYP3A substrate and inhibitor and is a P-glycoprotein (P-gp) substrate. Hence, itraconazole plasma concentration can be nonlinear with dose, and caution should be used in coadministration with other drugs.

Recently, we have described the preparation and characterization of hydroxybutenyl-β-cyclodextrin (HBenBCD, Figure 1). Hydroxybutenyl-β-cyclodextrin is an amorphous white solid (Tg ca. 210°C) that is highly soluble in water (>500 g/L) and in organic solvents such as polyethylene glycol 400 (>400 g/L). In this account, we describe our investigations of the solubilization of itraconazole using HBenBCD and HPBCD as complexing agents. We also describe the preparation of solid inclusion complexes of itraconazole with HBenBCD and compare the dissolution behavior of these complexes to the parent drug, itraconazole, and to the commercial Sporanox® formulations. Additionally, we report our preclinical results after intravenous and oral administration of itraconazole:HBenBCD and Sporanox® formulations to male Sprague–Dawley rats. Our goals were to directly compare the solubility and dissolution profiles obtained with these formulations, and to determine the correlation between in vitro and in vivo results. We were particularly interested in probing the effects of CD structure, formulation, and food on absorption, metabolism, elimination, and oral bioavailability of itraconazole in this animal model.

**EXPERIMENTAL**

**General Methods**

Hydroxybutenyl-β-cyclodextrin (Molar Substitution = 4.7) was prepared according to previously described methods. Hydroxypropyl-β-cyclodextrin (MS = 4.5) was obtained from Aldrich (Milwaukee, WI) and used without any further purification. All CD derivatives were dried at 10–15 mmHg at room temperature for 14–60 h prior to use. Crystalline itraconazole was obtained from Apin Chemicals and characterized prior to use. Sporanox® (Janssen Pharmaceutica N.V.) intravenous solution, oral solution, and capsules were obtained by prescription and each dosage form was used without modification. Hydroxyitraconazole and itraconazole-d₃ were obtained from SynFine (Ontario, Canada). Ethylenediaminetetraacetic acid trisodium salt hydrate (EDTA) was obtained from Aldrich.

Water was filtered through a Milli-Q Water System equipped with a 0.22 µm sterile biofilter (www.millipore.com/catalogue.nsf/docs/C7658) and had very low total organic and pyrogen content and low ionic strength. The water was not degassed prior to use and typically had a pH of 4.7. Buffers were prepared using Millipore water. All glassware and tools used in complex preparation were washed extensively with water and then with absolute EtOH and dried at 115°C for 1–24 h.

Biorelevant test media (Tab. 1), used in both solubility and dissolution experiments, were selected to represent the different physicochemical conditions in the upper human GI tract that
Table 1. Biorelevant Test Media Used for Solubility Studies and Dissolution Experiments

<table>
<thead>
<tr>
<th>Test Medium (Water)</th>
<th>pH</th>
<th>mOsmol/kg</th>
<th>GI Segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGFsp USP 28</td>
<td>1.2</td>
<td>182</td>
<td>Stomach</td>
</tr>
<tr>
<td>SGFsp mod</td>
<td>1.8</td>
<td>118</td>
<td>Stomach</td>
</tr>
<tr>
<td>Acetate buffer</td>
<td>5.0</td>
<td>10</td>
<td>Stomach</td>
</tr>
<tr>
<td>Blank FaSSIF</td>
<td>6.5</td>
<td>266</td>
<td>Upper small intestine fasted</td>
</tr>
<tr>
<td>FaSSIF</td>
<td>6.5</td>
<td>273</td>
<td>Upper small intestine fasted</td>
</tr>
<tr>
<td>Blank FeSSIF</td>
<td>5.0</td>
<td>622</td>
<td>Upper small intestine fed</td>
</tr>
<tr>
<td>FeSSIF</td>
<td>5.0</td>
<td>646</td>
<td>Upper small intestine fed</td>
</tr>
<tr>
<td>Acetate buffer</td>
<td>4.5</td>
<td>291</td>
<td>Duodenum</td>
</tr>
<tr>
<td>SIFsp USP 28</td>
<td>6.8</td>
<td>100</td>
<td>Small intestine</td>
</tr>
</tbody>
</table>

*Contains physiological concentrations of bile components.

may influence solubility and dissolution of itraconazole. The biorelevant media were prepared according to standard procedures and appropriate precautions were taken to ensure their integrity. The pharmacopoeial buffers simulated gastric fluid without pepsin; SGFsp USP 28 pH 1.2, simulated intestinal fluid without pancreatin; SIFsp USP 28 pH 4.5 and acetate buffer USP 28 pH 4.5 represent average pH conditions in the human stomach, duodenum, and jejunum and are commonly used to determine the drug solubility in terms of BCS classification according to the FDA guideline. The biorelevant test media fasted/fed state simulated intestinal fluid (FaSSIF and FeSSIF) and their corresponding blanks were used to determine solubility under upper small intestinal pH conditions in the fasted and fed state, and also to determine whether amphiphilic bile components including bile salts and lecithin, the concentration of which increase following a meal, influence drug in vitro solubility.

Since dissolution of weak bases such as itraconazole under gastric conditions is crucial for their bioavailability, it was important that we adequately simulate gastric conditions. The standard SGFsp addresses many gastric juice qualities, but there are some aspects that may be optimized. For example, most gastric pH studies, even in young healthy volunteers, indicate that gastric pH usually lies in the range 1.5–2.5, with an across-the-board average of about 1.8. Therefore, for very poorly soluble weak bases, the dissolution results in standard SGFsp are likely to overestimate the in vivo rate. Based on these considerations, a modified SGFsp with a pH of 1.8 was included to simulate the average gastric pH in healthy individuals. Furthermore, recently it has been shown that elevated gastric pH caused by achlorhydria or the intake of proton pump inhibitors and H₂ antagonists can result in compromised absorption of poorly soluble weak bases. For this reason acetate buffer (pH 5.0) was included to simulate gastric conditions of patients with elevated gastric pH.

Solubility Studies

Solubility measurements were made using a modified version of the traditional shaking flask method [Buchanan CM, Buchanan NL, unpublished work]. To each well of a 2 mL 96-well polypropylene mixing plate was added excess drug (ca. 5–10 mg) or, in the case of solubility testing in biorelevant media, itraconazole:H-BenBCD (12.2 wt% itraconazole). To each well of the preloaded 96-well mixing plate was added 300–500 µL of water, buffer, or biorelevant media (no CD) or the appropriate CD solution. Each determination was made in triplicate. The blanks were used to determine the intrinsic solubility (Sᵢ) of drug in that solution, and the wells containing the CD solutions were used to determine the solubility of drug due to CD (Sᵢ). After addition of the stock solutions, the plate was sealed using aluminum foil with a nonvolatile adhesive on one surface. The plate was placed on a rotary shaking plate (Helidolph Titramax 1000) and the plate was shaken at 800–1200 rpm at 23 or 37 ± 2°C for 48–72 h. Preliminary experiments showed that less than 24 h was required to reach equilibrium (i.e. drug concentration plateaued and did not change with time). Longer mixing times were used both for convenience and to insure that we were well above the time required to reach equilibrium. During the mixing period, the plate was inspected to ensure that each well contained undissolved, excess drug. Drug was added as necessary to maintain an excess.

Following the mixing period, the solutions in each well were transferred to the corresponding wells of a 96-well 2 mL multi-screen filter plate using a multi-channel pipette. The bottom of each well was a hydrophilic membrane. The filter plate was placed on top of a vacuum manifold and the solutions were filtered at ca. 20 mm Hg into the corresponding wells of a 2 mL storage plate. The duration of the filtration period was typically no longer than 60 s. The storage plate was then sealed with a silicon mat and samples were removed for analysis as appropriate.

The drug content in each well was determined by UV spectroscopy using a SpectraMax Plus 384 Molecular Devices multi-well plate reader. Typically, 10–20 μL of drug solution was transferred to the corresponding well of a 96-well measurement plate (UV-STAR plates from Greiner with a spectral range of 190–400 nm) and, if necessary, diluted with 1/1 water/ethanol so that the absorbance was in the linear response range. It is important to note that in these experiments, if the drug has low absorptivity and if the drug concentration is low, it is necessary to subtract absorbance contributions from the measurement plate and from the buffer from the measured absorbance. Absorbance was converted to drug concentration using the appropriate absorptivity for the drug. The drug concentrations were then exported to the appropriate software package for final analysis. Additionally, the final pH of each well was measured to insure that the pH had not drifted significantly due to lack of buffering capacity.

Preparation of Itraconazole:HBenBCD Complexes

The exact methods useful for the preparation of drug:CD complexes are highly dependent upon the drug, CD, and how the formulation may be used. The following are procedures used to prepare the itraconazole:HBenBCD complexes described in this manuscript. These procedures parallel published procedures for preparing itraconazole:HPBCD complexes.

**Itraconazole:HBenBCD Complex for IV Administration**

A 58.8 wt% aqueous HBenBCD solution was prepared by dissolving 10 g of previously dried HBenBCD in 7.0 g of water. A second solution was prepared by first adding 900 μL of concentrated HCl to 10 mL of propylene glycol (dried over 4 Å molecular sieves). To a glass vial containing 250.6 mg of itraconazole was added 1000 μL of the propylene glycol/HCl solution. The mixture was vortexed until a homogeneous solution was obtained.

The itraconazole/propylene glycol solution was then slowly added to the HBenBCD aqueous solution with vigorous mixing giving a homogeneous solution. The pH of the solution was then adjusted to pH 4.5 by the slow addition of 0.1 M NaOH with vigorous mixing. The resulting solution was then filtered through a 0.45 μm filter into 25 mL volumetric flask and the solution was diluted to 25 mL with water. The final formulation contained 40 wt% HBenBCD, 3.6 wt% propylene glycol, and 0.78 wt% itraconazole at pH 4.5.

**Itraconazole:HBenBCD Powder Complex Prepared in Water/EtOH for Oral Administration**

A 55.6 wt% aqueous HBenBCD solution was prepared by dissolving 5 g of previously dried HBenBCD in 4 g of water. A second solution was prepared by first adding 900 μL of concentrated HCl to 10 mL of absolute ethanol. To a glass vial containing 700 mg of itraconazole was added 2000 μL of the ethanol/HCl solution. The mixture was vortexed until a homogeneous purple solution was obtained.

The itraconazole/EtOH solution was then slowly added to the HBenBCD aqueous solution with vigorous mixing giving a homogeneous pale tan solution. The pH of the solution was then adjusted to pH 2.0 by the slow addition of 0.1 M NaOH with vigorous mixing. The resulting solution was then filtered through a 0.45 μm filter into a freeze dry flask. Freeze drying at ca. −45 °C, 130 mTorr gave 5.45 g of a white powder containing 12.6 wt% itraconazole.

**Itraconazole:HBenBCD PEG400 Solution**

A 41 wt% itraconazole-HBenBCD-PEG400 solution was prepared by dissolving 5 g of previously dried HBenBCD in 7.2 g of PEG400 (dried over 4 Å molecular sieves). A second solution was prepared by first adding 900 μL of concentrated HCl to 10 mL of propylene glycol (dried over 4 Å molecular sieves). To a glass vial containing 701 mg of itraconazole was added 2400 μL of the propylene glycol/HCl solution. The mixture was vortexed until a homogeneous solution was obtained.
The itraconazole/propylene glycol solution was then slowly added to the HBenBCD PEG400 solution with vigorous mixing giving a homogeneous solution. The solution was then adjusted to pH 2.5 by the slow addition of propylene glycol containing 9.2 wt% KOH with vigorous mixing. The resulting solution was completely clear and did not require filtration. The final formulation contained 25 wt% HBenBCD, 38 wt% propylene glycol, and 3.75 wt% itraconazole in PEG400 at pH 2.5.

Dissolution Studies

Standard dissolution studies were conducted at 37°C with a stirring speed of 50 rpm combined with 900 mL of test medium according to USP 28-NF 23 711.35 For these studies, the experimental solid or PEG 400 liquid fill formulations were filled into hard shell Torpac lock ring gelatin capsules (Size #1 or #0). In these experiments, the amount of itraconazole:CD complex or Sporanox® beads utilized in each dissolution vessel corresponded to 27 ± 5 mg of itraconazole. The apparatus used for the testing was a Varian VK 7000 dissolution tester. The pH values used for the experiments were selected on the basis of the pH normally found in the stomach or upper and lower GI tracts of humans.

Dissolution studies in biorelevant media were performed with a mini-paddle apparatus (modified DT 606 HH, Erweka, Heusenstamm, Germany). This mini-paddle was based on the USP paddle setup but scaled down exactly 1/3 with respect to the one-dimensional parameters which results in half of the filling volume. Preliminary experiments have shown that this test system requires smaller sample sizes and smaller volumes of media but has the same reliability and predictivity as the standard test apparatus.36

The test was designed to correspond with current pharmacopeial and BCS conform dissolution tests, particularly with the one recently proposed as a standard test for immediate release solid oral dosage forms.37,38 The cited proposal recommends use of the paddle apparatus with a stirring speed of 75 rpm combined with 500 mL of test medium. As the use of the mini paddle apparatus allowed reducing the sample size to half of the dose and half of the standard volume of medium, 250 mL of test medium was used for each experiment. Experiments were run at 37 ± 0.5°C applying a stirring speed of 100 rpm which has been shown to correspond to 75 rpm in the standard paddle setup.36 In these experiments, the test dose was 50 mg of itraconazole or the corresponding amount of itraconazole:HBenBCD complex (404 mg), which is equivalent to a 100 mg dose in a standard paddle apparatus. Samples (2.5 mL) were removed after 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, and 240 min and each was replaced with the same volume of blank, prewarmed medium. Experiments were run in triplicate and results expressed as mean % dissolved at the given sampling time.

Following appropriate dilution, samples were analyzed by HPLC. The HPLC system consisted of a LaChrom L-7100 pump, a LaChrom L-4250 UV-VIS Detector, a LaChrom L-2000 auto sampler, and EZChrom Elite Chromatography Data System software (Merk Hitachi, Darmstadt, Germany). Before analysis, all samples were diluted 1:5 using a mixture of methanol:30 mM hydrochloric acid 75:25. The analyses were performed on a Lichrocart® RP-18, 5μ, 125×4 m column (Merck, Darmstadt, Germany), using a mixture of acetonitrile:15 mM phosphate buffer pH 3.0 75:25 as mobile phase. Flow rate was set at 1.2 mL/min, resulting in elution of itraconazole at 3 min. The amount of released drug was determined using a wavelength of 260 nm.

Animals

The in-life portion of the study was conducted at RCC Ltd (Toxicology, CH-4452 Itingen, Switzerland). Male Sprague—Dawley rats (weight, 317 ± 23 g) were obtained from RCC Ltd (Laboratory Animal Services, CH-4414 Füllinsdorf, Switzerland). Rats were housed individually in Makrolon type-3 cages with wire mesh tops and standardized softwood bedding (Lignocel Schill AG, CH-4132 Muttenz, Switzerland) prior to dosing. The room was air-conditioned with 10–15 air changes per hour, and was maintained at 22 ± 3°C and a relative humidity between 30% and 70%. The rats were subjected to 12 h fluorescent light/12 h dark cycles with music during the light period. The animals were allowed free movement and access to water. Access to food was managed as described in the study design (vide infra).

Pharmacokinetic Study Design

The study design for itraconazole:CD formulations is summarized in Table 2. The dose and the
Table 2. Dosing Groups for the Pharmacokinetic Study Involving Itraconazole (20 mg/kg, n = 4) Formulated With Cyclodextrins

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>Sporanox® injection solution. Rats were administered 3.3 mL/kg body weight</td>
</tr>
<tr>
<td>IV</td>
<td>HBenBCD aqueous solution (Sporanox® containing HBenBCD) (40 wt%). Rats were</td>
</tr>
<tr>
<td>IV</td>
<td>administered 2.5 mL/kg body weight</td>
</tr>
<tr>
<td>IV</td>
<td>Capsule filled with itraconazole:HBenBCD powder. Each rat was given two</td>
</tr>
<tr>
<td>IV</td>
<td>capsules (ca. 15 mg powder per capsule)</td>
</tr>
<tr>
<td>Oral</td>
<td>Capsule liquid containing itraconazole, HBenBCD (33 wt%). PEG400, and PC.</td>
</tr>
<tr>
<td>Oral</td>
<td>Rats were given 0.53 mL/kg body weight</td>
</tr>
</tbody>
</table>

Blood sampling times were based on a dosing tolerance prestudy (data not shown). Groups 1 and 2 were given intravenous doses of a Sporanox® injection solution (contains HPBCD) and a HBenBCD aqueous solution (formulated identically to the Sporanox® injection solution). Groups 3 and 4 were administered by aqueous oral gavage and, as they are formulated identically and given to animals with the same dietary state, allow the direct comparison of itraconazole:HBenBCD and itraconazole:HPBCD oral solution formulations. Group 5 (fed) was administered Sporanox® bead (no CD) filled capsules; Sporanox® beads were removed from the prescription capsule and placed in #9 gelatin capsules (each capsule will hold a maximum of ca. 19 mg of beads) for animal dosing. Based on analysis of the entire content of several Sporanox® capsules, the itraconazole loading on the beads was 22.3 wt%. Groups 6 (fed) and 7 (fasted) were given the same itraconazole:HBenBCD powder formulation filled into gelatin capsules. Group 8 was given a solution of HBenBCD and itraconazole in PEG400 and propylene glycol. Groups 6 and 7 probe the effect of the dietary state of the animals on oral bioavailability when given the same formulation while groups 7 and 8 compare two different formulations given to animals in the fasted dietary state.

Groups of four male Sprague—Dawley rats were administered different formulations of itraconazole (20 mg/kg) by intravenous infusion via the tail vein at 0.3 mL/min, by oral gavage, or by oral administration of solids filled capsules (#9 gelatin capsules, www.torpac.com). Animals were allowed free movement and access to water at all times and all were fasted for at least 8 h prior to dosing. Animals in the two fed groups (groups 5 and 6), received 1 mL of homogenized commercial rodent chow in pH 1.8 water by oral gavage 30 min prior to itraconazole dosing. The remaining groups received 0.5 mL pH 1.8 water by oral gavage 30 min prior to itraconazole dosing. Free access to food was restored to all animals 5 h after dose administration.

Blood Sampling

Serial blood samples (220 μL) were collected from each animal through a catheter inserted into the jugular vein into tubes containing K3 EDTA by automated blood sampling using a DiLab AccuSampler. Samples were taken prior to the dose
and at 0.25, 0.5, 1.5, 3, 4, 5, 9, 12, 24, and 48 h postdose (intravenous treatment) or 1, 2, 3, 4, 5, 6, 9, 12, 24, and 48 h postdose (oral treatment). Blood was replaced by an equivalent volume of intraperitoneal saline after each draw. All animals were euthanized following terminal blood collection. Plasma was separated by centrifugation under refrigeration (2–8°C) for 10 min then each plasma sample was collected and placed into an individual well of a 96-well storage plate. The storage plate was kept at −80 ± 10°C until analysis.

Analysis of Plasma Samples for Itraconazole and Hydroxyitraconazole

A Waters (liquid chromatography/mass spectrometry/mass spectrometry; LC/MS/MS) system (Milford, MA) was employed for the analyses. The LC was an Acquity Ultra Performance LC (UPLC) and the triple quadrupole mass spectrometer was a Quattro Micro. The eluent was introduced into the electrospray source and the instrument operated in the positive ion multiple reaction monitoring (MRM) mode employing argon as a collision gas. The following MRM transitions were monitored: m/z 705.3—>392.0, m/z 708.3—>395.0, and m/z 721.3—>408.0 for itraconazole, itraconazole-d₃ (internal standard), and hydroxyitraconazole, respectively. The ratio of all solvent mixtures is listed in volume:volume. The LC separations were performed with a Varian column (MonoChrom C18, 3 μm, 50 mm × 2.0 mm). Solvent A was a mixture of 1000 mL of aqueous ammonium formate (10 mM, pH adjusted to ca. 3.5 with formic acid) and 30 mL of 1:1 methanol:acetonitrile. Solvent B was 1:1 methanol:acetonitrile. The column temperature was ca. 50°C, the sample temperature was ca. 4°C, and the injection volume was 5 μL. The following linear gradient was employed for the separation: 70% A for 0.2 min, 0.4 mL/min; 25% A at 0.3 min, 0.4 mL/min; 17% A at 1.7 min and hold to 2.1 min, 0.4 mL/min; 0% A at 2.2 min and hold to 3.0 min, 1.6 mL/min; and 70% A at 3.01 min and hold to 4.0 min, 0.4 mL/min. The elution times for itraconazole, itraconazole-d₃ (internal standard), and hydroxyitraconazole were 1.61, 1.61, and 1.27 min, respectively. The solvent flow was diverted from the mass spectrometer before 1.1 min and after 1.9 min. The chromatographic conditions separated the analytes from the phospholipids that often cause significant matrix ionization effects. The response for the itraconazole was essentially the same in the blank study plasma compared to the plasma purchased from Bioreclamation for calibration and quality control. However, the response for the hydroxyitraconazole was ca. 5–20% less in the blank study plasma compared to the purchased plasma. This effect was minimized by mixing 25 μL of sample with 75 μL of Bioreclamation plasma for all analyses.

Briefly, 25 μL of the study plasma sample was mixed with 75 μL of Bioreclamation plasma and 25 μL of 1:1:1 methanol:water:acetonitrile in a 96-well plate and vortexed for 4 min. Proteins were precipitated using 250 μL of 1:1 methanol:acetonitrile containing the internal standard and vortexed for 4 min. The samples were centrifuged at 1509g for 10 min. The plasma supernatants were directly analyzed using an auto-sampler syringe setting that collected above the protein pellet.

All the itraconazole calibrators and quality samples measurements met the criteria set in the FDA protocol. All the hydroxyitraconazole values (measured by external standard) met the criteria set in the FDA protocol except groups 7 and 8. The instrument response drifted too much for these groups, so the QC samples were needed to adjust the calibration.

Pharmacokinetic Analysis

PK parameters were determined using standard, noncompartmental techniques. The areas under
the plasma concentration–time curves (AUC) from time zero to 48 h (AUC_{0–48h}) were calculated using the linear and logarithmic trapezoid methods during the rising and declining plasma level phases, respectively. Maximum drug concentration in plasma (C_{max}) and the time to reach this concentration (T_{max}) were determined from the plasma concentration–time curves. Oral bioavailabilities were determined using the AUC from the IV and oral doses.

During the in-life portion of this study, blood samples were missed due to pressure drops in the catheter. For reasons of animal safety, the DiLab AccuSampler would not take a sample if the pressure in the catheter was less than or equal to 8 kPa. Due to the size (eight groups) and length of the study (48 h), individuals were not present at certain times to manually remove blood samples. This was unfortunate as in practice, the number of animals sampled (n) was not constant at all time points (vide infra). As this was intended as a preliminary study with small groups of animals, the loss of blood samples rendered statistical analysis of the PK data impossible. For this reason, we report mean PK values and provide the number of blood samples (n) and standard deviations separately in the supplementary material section of this manuscript.

RESULTS AND DISCUSSION

Solubility Studies

Figure 2 shows the solubility of itraconazole in water and in three buffers containing 10 wt% HBenBCD and HPBCD obtained in initial screening studies at 23°C. For comparative purposes, the solubility of itraconazole in the absence of CD is also provided. Clearly, significant differences exist between the CDs in their ability to solubilize itraconazole. In water, HBenBCD increased the solubility of itraconazole from 30 µg/mL (S_0) to 515 µg/mL (S_t/S_0 = 17.2). For HPBCD, the observed S_t/S_0 was 3.8. Similar differences between HBenBCD and HPBCD were observed with the buffer solutions. It is interesting to note that in the case of the blanks (no CD), none of the buffers offered significant increases in itraconazole solubility relative to water. Note that the current formulation of itraconazole used clinically for intravenous administration contains itraconazole/HPBCD complex in water/propylene glycol (Sporanox®). As this data shows, HBenBCD provides a 4.5 x increase in itraconazole solubility relative to HPBCD under identical conditions.

Figure 3 shows the equilibrium solubility of itraconazole versus varying concentrations of HBenBCD and HPBCD in pH 3 phosphate buffer. In the case of HBenBCD, the equilibrium solubility of itraconazole at variable HBenBCD concentrations in water is also included. We observed no significant difference in itraconazole solubility in water versus pH 3 phosphate buffer when using HBenBCD as the complexing agent. As can be seen from Figure 3, HBenBCD provided a significant increase in itraconazole solubility relative to HPBCD. At ca. 25 wt% CD, the S_t/S_0 ratio for HBenBCD was 132 which was 4 x greater.
than that observed for HPBCD. Interestingly, the solubility of itraconazole in the HBenBCD and HPBCD solutions was not linear with [CD] over the examined concentration range. The curvature of these solubility isotherms can be classified as type Aₚ suggesting the formation of higher-order complexes. Although stability constants can be experimentally determined for these higher-order complexes ($K_{1:n}$), this effort was judged to be outside the scope of the current work.

Following the preliminary solubility screening studies, we examined the solubility of itraconazole formulated with HBenBCD in biorelevant media at 23°C and at 37°C (Fig. 4). In the absence of HBenBCD, the amount of itraconazole dissolved was below the detection limit in most of the relevant test media (data not shown). In SGFsp pH 1.2, itraconazole solubility was 0.22 μg/mL at 37°C. Increasing the pH of SGFsp to 1.8 at the same temperature decreased the solubility of itraconazole to 0.10 μg/mL. In contrast, a tremendous improvement in itraconazole solubility was observed in the presence of HBenBCD in all test media. As expected for a weakly basic drug, the solubility of the HBenBCD:itraconazole complex was greatest under simulated gastric conditions (ca. 10000-fold at pH 1.2 and ca. 5000-fold at pH 1.8). The amounts of dissolved drug and HBenBCD were so great that they resulted in a remarkable increase in solution viscosity, making it impossible to determine the infinite solubility in these media. As would be expected, as the pH of the medium approached the $pK_a$ of itraconazole the solubility of the drug in presence of HBenBCD was diminished but still far better (in the range of 40–70 μg/mL) than in the absence of HBenBCD. Comparison of the solubility data in water, FaSSIF, and FeSSIF reveals that there are no significant differences in the solubility of itraconazole in these media when HBenBCD was present. Furthermore, comparing data in FaSSIF, and FeSSIF with and without bile components, it is likely that bile components moderately increase the solubility of itraconazole when HBenBCD is present. This observation would suggest that competitive complexation of bile salts with HBenBCD is not a major issue. Finally, it is worth noting that there were no significant differences in itraconazole solubility at 23°C versus 37°C in any of the media examined; due to this observation, we did not complete the 23°C series with FaSSIF and FeSSIF. High throughput screening of drug solubility is operationally much simpler at 23°C.

**Dissolution Studies**

The dissolution profiles obtained from the standard USP 28 method at pH 1.2 and 4.5 for itraconazole formulated with HBenBCD in powder filled gelatin capsules, are provided in Figure 5. Also included in Figure 5 are the profiles for the capsule form of Sporanox® (no CD, obtained by prescription). We elected to use the capsule form of Sporanox® rather than an itraconazole:HPBCD complex since we planned to use the capsule form of Sporanox® in our PK study.
Dissolution of itraconazole from the capsules filled with itraconazole:HBenBCD complex was very rapid at pH 1.2 (ca. 100% in 15 min). Precipitation of itraconazole was not observed over the lifetime of the experiment. Release of itraconazole from the Sporanox® formulation was slower. After 15 min, ca. 36% of the itraconazole was dissolved and 100% dissolution was not obtained until ca. 3 h. At pH 4.5, the itraconazole:CD formulations again provided for a more rapid release and more complete (ca. 40%) dissolution of itraconazole relative to the Sporanox® formulation (ca. 6% dissolution).

As we have shown previously with tamoxifen, the solubility of HBenBCD in water-soluble organic solvents allows the development of liquid fill formulations with selected drugs. As the CD and drug do not form complexes in organic media, the success of this approach in a clinical setting depends upon the rate of complexation of the drug with HBenBCD being faster than that of precipitation of the drug upon exposure to the aqueous medium of the gut. Figure 6 compares the dissolution profile of itraconazole:HBenBCD formulated in PEG400/propylene glycol to that of an itraconazole:HBenBCD powder complex in gelatin capsules. Both formulations provide for rapid dissolution of itraconazole at pH 1.2 and 4.5. However, it should be noted that dissolution of itraconazole from the powder formulation was more complete than from the liquid formulation at both pH 1.2 (ca. 100% vs. 90%) and pH 4.5 (ca. 40% vs. 30%).

Figure 7 shows the dissolution profiles of itraconazole (formulated with HBenBCD) in biorelevant media obtained using the mini-paddle dissolution apparatus. The results obtained in these dissolution studies were in good agreement with the observations made in the solubility studies. In SGFsp pH 1.2, a rapid release of the entire dose was observed. The resulting drug solution was stable over the test duration of 4 h indicating that the drug would not precipitate in the stomach. Increasing the gastric pH to a value of 1.8 also resulted in rapid drug release but, as expected, the maximum dissolution was ca. 45%. At pH 6.8, the least favorable pH for dissolution of the poorly soluble weak base, minimal dissolution was observed with only 1 wt% itraconazole being dissolved after 4 h. In FaSSIF, the biorelevant dissolution medium simulating the physiological conditions in the upper small intestine in the fasted state, a maximum release of ca. 7% itraconazole was observed; this itraconazole concentration was maintained over the whole experiment. Comparison of the dissolution profile obtained in FaSSIF to the blank buffer (ca. 2% drug released), suggests that the presence of physiological concentrations of bile components may facilitate dissolution of itraconazole. Likewise, dissolution of itraconazole in FeSSIF, the biorelevant medium simulating fed state conditions in the upper small intestine, was slightly
more complete than that obtained in the corresponding blank buffer. We did not observe significant differences in either the dissolution rate or the total amount of itraconazole released between the simulations of fasted (FaSSIF) and fed (FeSSIF) states.

Pharmacokinetic Study

Based on the knowledge obtained from the solubility and dissolution studies on the itraconazole formulations, we initiated a preclinical trial with the study design (vide supra) shown in Table 2.

Figure 8 provides plasma concentration–time curves for itraconazole and hydroxyitraconazole after intravenous doses of Sporanox® injection solution (group 1) and itraconazole:HBenBCD aqueous solution (group 2). PK parameters are summarized in Table 3. The most notable feature of the data presented in Figure 8 is the apparent secondary absorption of itraconazole at ca. 12 h which is also reflected in the hydroxyitraconazole plasma concentration. This observation suggests hepatic recirculation of itraconazole mediated by HBenBCD which is surprising as the literature indicates that CD derivatives are eliminated via the kidneys. The net result is that the AUCs for itraconazole and hydroxyitraconazole obtained after administration of the itraconazole:HBenBCD aqueous solution are slightly greater (ca. 18%) than those obtained with the Sporanox® injection solution (Tab. 3).

Figure 9 displays plasma concentration versus time profiles for itraconazole and hydroxyitraconazole after dosing of Sporanox® (contains HPBCD) and itraconazole:HBenBCD oral solutions. The itraconazole plasma concentration versus time profiles for these formulations are similar. Following a rapid increase in itraconazole plasma concentration, itraconazole plasma concentrations begin to level off or decline between ca. 2-4 h followed by a secondary increase in plasma concentrations between ca. 5-6 h (Tab. 3). In the case of the itraconazole:HBenBCD oral solution, the second rise in itraconazole plasma concentrations are larger than that observed for the itraconazole:HPBCD oral solution. The net effect is that the itraconazole AUC for the itraconazole:HBenBCD oral solution (14526 ng·h/mL) is larger than the Sporanox® (10330 ng·h/mL) oral solution. The plasma concentration versus time profiles for hydroxyitraconazole are similar to those observed for itraconazole. In the case of the itraconazole:HBenBCD oral solution, the secondary absorption is quite distinct leading to a T_max of ca. 6 h (Tab. 3). In contrast, with the Sporanox®, T_max for hydroxyitraconazole is less well defined as the rate of absorption—elimination of hydroxyitraconazole is nearly equal in the 3–6 h time frame. The AUC of hydroxyitraconazole from administration of the itraconazole:HBenBCD oral solution (13823 ng·h/mL) is larger than those from the Sporanox® (10425 ng·h/mL) oral solution.

Figure 10 shows the plasma concentration versus time profiles for itraconazole and hydroxyitraconazole after oral dosing with capsules of Sporanox® beads (group 5, no CD, fed animals), itraconazole:HBenBCD powder (groups 6 and 7, fed and fasted animals), and with a itraconazole:HBenBCD-PEG400-PG liquid (group 8, fasted animals). In the case of Sporanox® beads, the itraconazole AUC was 3533 ng·h/mL and the observed T_max was 4 h (Tab. 3). Fed animals dosed orally with itraconazole:HBenBCD powder had an itraconazole AUC of 6209 ng·h/mL, with T_max

Figure 8. Plasma concentration–time curves for itraconazole and hydroxyitraconazole after intravenous doses of Sporanox® injection solution (group 1) and itraconazole:HBenBCD aqueous solution (group 2).
Table 3. Pharmacokinetic Parameters for Itraconazole (ITZ) and Hydroxyitraconazole (HITZ)

<table>
<thead>
<tr>
<th>Group</th>
<th>C_{max} (ng/mL)</th>
<th>T_{max} (h)</th>
<th>AUC_{0-48h} (ng·h/mL)</th>
<th>% F (0–48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporanox® IV (G1)</td>
<td>2951.0</td>
<td>0.5</td>
<td>2775.1</td>
<td>—</td>
</tr>
<tr>
<td>HBenBCD IV (G2)</td>
<td>2755.0</td>
<td>0.3</td>
<td>3268.7</td>
<td>32344</td>
</tr>
<tr>
<td>Sporanox® oral solution (G3)</td>
<td>443.7</td>
<td>5.0</td>
<td>10330</td>
<td>31.6</td>
</tr>
<tr>
<td>HBenBCD oral solution (G4)</td>
<td>620.9</td>
<td>5.0</td>
<td>14525</td>
<td>44.4</td>
</tr>
<tr>
<td>Sporanox® capsules (G5)</td>
<td>228.3</td>
<td>5.0</td>
<td>3533</td>
<td>10.8</td>
</tr>
<tr>
<td>HBenBCD capsules fed (G6)</td>
<td>326.3</td>
<td>9.0</td>
<td>6209</td>
<td>19.0</td>
</tr>
<tr>
<td>HBenBCD capsules fasted (G7)</td>
<td>329.3</td>
<td>4.0</td>
<td>6607</td>
<td>20.2</td>
</tr>
<tr>
<td>HBenBCD LF gavage (G8)</td>
<td>175.0</td>
<td>6.0</td>
<td>3109</td>
<td>9.5</td>
</tr>
</tbody>
</table>

apparently shifted to 9 h. However, close inspection of Figure 10 shows that the apparent shift in T_{max} may be due to a secondary absorption phase; there is an earlier maximum at 4 h. Fasted animals dosed orally with the same itraconazole:HBenBCD powder had an AUC of 6607 ng·h/mL, with a T_{max} of 4 h. That is, there are apparently no significant food effects on the PK parameters after dosing rats with itraconazole: HBenBCD powder. It is interesting to note that, while both the C_{max} and the AUC for the HBenBCD powder formulations were ca. two-times greater than the Sporanox® beads formulation, T_{max} for the two formulations were the same.

Figure 9. Plasma concentration–time curves for itraconazole and hydroxyitraconazole after dosing of rats with aqueous oral gavages of Sporanox® solution (contains HPBCD) and itraconazole:HBenBCD solution.

Figure 10. Plasma concentration–time curves for itraconazole and hydroxyitraconazole after oral dosing of rats with capsules of Sporanox® beads (no CD, fed animals), itraconazole:HBenBCD powder (fed and fasted animals), and with an itraconazole-HBenBCD-PEG400-PG liquid (fasted animals).
In general, CD-containing drug formulations tend to have reduced \( T_{\text{max}} \) relative to drug formulations that do not contain CDs.\(^4\)

In contrast to groups 6 and 7 (HBenBCD powder formulations), dosing of animals with an itraconazole-HBenBCD-PEG400-PG liquid (group 8) resulted in a lower AUC (3109 ng \( \cdot \) h/mL) and a lower \( C_{\text{max}} \) (175 ng/mL). This was in contrast to similar liquid fill formulations of tamoxifen-HPBCD which led to increased AUC and \( C_{\text{max}} \). Relative to group 4 (itraconazole:HBenBCD aqueous gavage), the AUC and \( C_{\text{max}} \) for group 8 were significantly lower; relative to group 5 (Sporanox® beads), the AUC and \( C_{\text{max}} \) for group 8 were equivalent.

The plasma concentration \textit{versus} time profiles for hydroxyitraconazole parallel those for itraconazole for groups 5–8. For example, a secondary absorption phase for hydroxyitraconazole was noted for group 6 (itraconazole:HBenBCD powder), as was also noted for itraconazole in that group.

Inspection of Table 3 reveals that the ratios of itraconazole/hydroxyitraconazole AUC after oral dosing are dependent upon the dosage forms. In the case of dosing with oral solutions of itraconazole:CD, the ratios of itraconazole/hydroxyitraconazole AUC were ca. 1.0. Fed animals dosed with the solid formulations had itraconazole/hydroxyitraconazole AUC ratios of ca. 0.6–0.8. Within these groups, group 7 (fasted animals dosed with itraconazole:HPBCD-HBenBCD powder) had the highest itraconazole/hydroxyitraconazole AUC ratio (0.78) while group 6 (Sporanox® beads) had the lowest AUC ratio (0.61). Group 8 (itraconazole:HBenBCD-PEG400-PG liquid), exhibited the lowest itraconazole/hydroxyitraconazole AUC ratio (0.4).

The oral bioavailability of itraconazole obtained with each dosage form is also provided in Table 3. The highest oral bioavailability was obtained with the oral solutions. Dosing of the Sporanox® liquid solution (group 3, contains HPBCD) gave an oral bioavailability of 32%. Dosing with the itraconazole:HBenBCD oral solution (group 4) resulted in an oral bioavailability of 44%, or 1.4-times that observed with the HPBCD. In the case of the itraconazole:HBenBCD powder filled capsules, the observed oral bioavailabilities were similar (19–20%) regardless of the dietary state of the animals, and were two-times that obtained by dosing with Sporanox® beads (no CD). The oral bioavailability obtained with the itraconazole:HBenBCD-PEG400-PG liquid dosage form was essentially the same as that obtained with the Sporanox® beads. The results obtained in this study are comparable to values obtained in other laboratories, which have found that the oral bioavailabilities of itraconazole obtained with Sporanox® oral and capsule form were 31–35% and 10%, respectively.\(^22,24\)

It is worth reiterating that the itraconazole:HBenBCD injection solution was prepared as closely as possible to the Sporanox® injection solution. Likewise, the itraconazole:HBenBCD oral solution mimics the Sporanox® oral solution. Hence, the results from the PK study represent a direct comparison of the two CDs formulated identically with itraconazole. In the case of the Sporanox® beads, the formulation is a solid dispersion of itraconazole (no CD) and a polymer phase is used to generate a carrier media dissolution profile where the codissolving polymer presumably acts to stabilize the formed supersaturated itraconazole solution via inhibition of nucleation and retardation of crystal growth.\(^6\) In contrast, the itraconazole:HBenBCD formulation is clearly a rapid release formulation with a distinctly different release profile as compared to the Sporanox® beads (Fig. 5). Hence, from a formulation point of view, one cannot directly compare the results from this PK study for the two systems as two different dissolution mechanisms are in operation. However, in the animal model utilized in this study, the rapid release formulation that contained a cyclodextrin (HBenBCD) resulted in a higher itraconazole oral bioavailability. The \( C_{\text{max}} \) and the AUC for the HBenBCD powder formulations were ca. two-times greater than the Sporanox® beads formulation (no CD), \( T_{\text{max}} \) for the two formulations were the same.

**CONCLUSIONS**

In this study, we have shown there are significant differences in the ability of HBenBCD and HPBCD to solubilize itraconazole. Although the substituents are attached to the same CD core, we observed as much as a 4.5 \times increase in the solubility of itraconazole with HBenBCD \textit{versus} HPBCD under identical experimental conditions. Clearly, the type of CD substituent can influence host:guest interactions in CD pharmaceutical formulations. Solubility and dissolution testing in biorelevant media indicated that no food effects should be observed after oral administration of itraconazole:HBenBCD complexes; this observa-
tion was validated in the PK study where no food effects were observed with HBenBCD solid dosage forms. Intravenous administration of itraconazole formulated with HBenBCD resulted in a higher AUC relative to the Sporanox® IV dosage form which contains HPBCD. When administered as an oral solution, the itraconazole:HBenBCD formulation provided enhanced oral bioavailability relative to the HPBCD formulation. When administered as a solid formulation, the itraconazole:HBenBCD formulation was superior to the Sporanox® solid formulation, which does not contain HPBCD, and to the HBenBCD liquid fill formulation. When the itraconazole:HBenBCD solid formulation was administered to the animals, the dietary state of the animals had no apparent effect on the observed oral bioavailability. Drug/metabolite ratios were found to be dependent upon the dosage form. The itraconazole/hydroxyitraconazole ratio decreased in the order of oral solutions (1.0) > solids (0.6–0.8) > liquid fill (0.4). This observation may suggest saturation of the CYP3A metabolic pathway with the CD containing oral solutions. One particularly significant observation was the increased secondary absorption of itraconazole after both intravenous and oral administration of HBenBCD formulations relative to the Sporanox® formulations, which merits further mechanistic investigation. Additional studies utilizing a larger number of animals are warranted to further probe the observations described in this account.

REFERENCES


46. The mean AUC, standard deviations, and the number of plasma samples for these groups are given in the supplementary material section.

