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When Interactions Between Bile Salts and Cyclodextrin Cause a Negative Food Effect: Dynamic Dissolution/Permeation Studies with Itraconazole (Sporanox®) and Biomimetic Media

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ABSTRACT

The marketed oral solution of itraconazole (Sporanox®) contains 40% (259.2 mM) of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). The obvious role of HP-β-CD is to solubilize itraconazole and to overcome its poor aqueous solubility that restricts its absorption.

In this study, we investigated the biorelevance of *in vitro* experiments by the influence of biomimetic media (containing bile salts and phospholipids) on the predicted itraconazole absorption from the commercial HP-β-CD-based Sporanox® solution. We performed phase-solubility studies of itraconazole and dynamic 2-step-dissolution/permeation studies using a biomimetic artificial barrier, Sporanox® solution, and fasted state simulated intestinal fluid (FaSSIF_V1).

Both FaSSIF_V1 and HP-β-CD increased the apparent solubility of itraconazole when used individually. In combination, their solubility-enhancing effects were not additive probably due to the competition of bile salts with itraconazole for the hydrophobic cavity of HP-β-CD. Our combined dissolution/permeation experiments indicated the occurrence of a transient supersaturation from Sporanox® upon two-step dissolution. Through systematic variation of bile salt concentrations in the biomimetic media, it was observed that the extent and the duration of supersaturation depend on the concentrations of bile salts: supersaturation was rather stable in the absence of bile salts and phospholipids. The higher the bile salt concentration, the faster the collapse of the transient supersaturation occurred, an effect which is nicely mirrored by reduced *in vitro* permeation across the barrier. This is an indication of a negative food effect, which in fact correlates well with what earlier had been observed in clinical studies for Sporanox® solution.

In essence, we could demonstrate that *in vitro* two-stage dissolution/permeation experiments using an artificial barrier and selected biomimetic media may predict the negative effects of the latter on cyclodextrin-based drug formulations like Sporanox® Oral Solution and, at the same time, provide a deeper mechanistic insight.

Introduction

Most new chemical entities in the pharmaceutical pipeline are poorly soluble and need to be formulated in a way that boosts their dissolution and/or solubility to enable a better absorption.[1,2] Cyclodextrins are commonly used with poorly soluble drug compounds due to their ability to increase solubility.[3] Cyclodextrin molecules are three-dimensional truncated cone structures characterized by a hydrophobic cavity and a hydrophilic external surface. The hydrophobic cavity allows molecules or moieties of appropriate size and lipophilicity to fit inside.[4] The formation of such complexes with small poorly water-soluble compounds is described by a concentration-dependent thermodynamic equilibrium by establishing and disrupting non-covalent interactions such as van der Waals interactions and hydrogen bonds. The formation of cyclodextrin-drug complexes can increase drug solubility, dissolution rate and bioavailability.[5]

Bile salts are amphipathic molecules produced by the liver or the intestinal flora; their concentration in the intestinal lumen varies depending on individuals and their feeding state. Their primary function is to emulsify lipids, and they control the osmotic flow between the liver and the bile capillaries, as well as signaling molecules in the

Abbreviations: FaSSIF_V1, Fasted State Small Intestinal Fluid; FeSSIF_V1, Fed State Small Intestinal Fluid; HP-β-CD, 2-Hydroxypropyl-β-Cyclodextrin; HPLC-UV PBS, High-Performance Liquid Chromatography incorporated with Ultra-Violet spectroscopy Phosphate Buffered Saline.

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fat and glucose metabolisms. In humans, the total bile salt concentration in the fasted state ranges between 1.4–8.1 mM; in the fed state, it increases to between 3.6 and 24.0 mM. Not only the concentration but also the molecular composition of the bile salt pool is subject to individual variability.[6-9]

When investigating the dissolution process of poorly soluble compounds regarding absorption, it is important to differentiate between molecularly dissolved and apparently dissolved fractions.[10] The term "molecularly dissolved" refers to dissolved free molecules exclusively surrounded by their hydration shell, while "apparently dissolved" refers to the total dissolved amount, including both molecularly dissolved compound and the fraction which is solubilized by, *e.g.*, micelles or cyclodextrins. Usually, only the molecularly dissolved species are considered to permeate across biological barriers.[11]

Since the mid-eighties, it has been recognized that bile salts have a high affinity for the lipophilic core of cyclodextrins and thus can displace lipophilic guest molecules[12] including drugs compounds,[13] in a concentration-dependent manner (up to their critical micelle concentration). The drug displacement is illustrated in Fig. 1. In the presence of both bile salts and cyclodextrins, a drug compound will appear in different dissolved states: included in micelles, complexed with cyclodextrins or molecularly dissolved. When bile salts interact with drug-cyclodextrin-complexes, they replace the drug in the cyclodextrin cavity, increasing the fraction of the non-complexed drug and thus enhancing permeation.[13] Only recently has it been demonstrated that the exchange between the dissolved states is dynamic. Bile salts can boost the free drug concentration to exceed the equilibrium solubility (supersaturation) and thus induce extraordinarily high permeation.[14]

Itraconazole is a lipophilic antifungal compound administered by the oral route: given its low solubility, the commercial formulations include Sporanox® Oral Solution and Sporanox® amorphous solid dispersion capsules. The oral solution uses 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) to maintain the drug in solution and therefore facilitate its absorption. Itraconazole is also weakly basic (pKa_1 at 3.70 and pKa_2 at 1.98), which translates to higher solubility at low pH, *e.g.*, in the stomach, leading to supersaturation of the compound upon transfer to the intestine.[15] *In vivo*, the Sporanox® Oral Solution has a slight negative food effect, while the amorphous solid dispersion shows an almost two-fold positive food effect.[16,17] However, a recent study using an advanced *in vitro* tool, Tiny-TIM, predicted a positive food effect for both the capsules and the oral solution.[18]

In the present study, we aimed to better elucidate the effect of the interactions between cyclodextrin, bile salts, and itraconazole when the Sporanox® Oral Solution is exposed to biomimetic media containing both bile salts and phospholipids, in order to evaluate in how far such media are biorelevant in terms of permeation and bioavailability. To this end, *in vitro* solubility and permeation experiments were carried out. The solubility experiments were conducted at different cyclodextrin concentrations and in Fasted State Simulated Intestinal Fluid (FaSSIF_V1) with different concentrations of the powder components (bile salts and phospholipids). In the permeation experiments, the Sporanox® Oral Solution was investigated in a 2-step approach mimicking the pH shift *in vivo* at relevant concentrations (powder components of lecithin and sodium taurocholate as in fasted state).

Materials and Methods

Chemicals and Equipment

Sodium phosphate dibasic, sodium phosphate monobasic and sodium chloride were purchased from Sigma-Aldrich (Brøndby, Denmark). Purified water was prepared with a Milli-Q® reference A+ water purification system from Merck KgaA (Darmstadt, Germany).

FaSSIF/FeSSIF/FaSSGF powder was purchased at Biorelevant.com Ltd. (London, United Kingdom). HP-β-CD was purchased from ABCR GmbH (Karlsruhe, Germany). Sporanox® Oral Solution was from Janssen-Cilag (Neuss, Germany) and purchased through a public pharmacy.

The employed equipment included: pH meter (Metrohm, Herisau, Switzerland), osmometer (KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany), Centrifuge 5804R (Eppendorf AG, Germany), High-Performance Liquid Chromatography with incorporated ultra-violet spectroscopy detector (HPLC-UV) (Waters Corporation, Milford, MA, USA), Permeapad® plates and Permeapad® membrane (InnoMe GmBH, Espelkamp, Germany) and side by side cells (PermeGear Inc., Hellertown, PA, USA)

Media Preparation

30 mM phosphate buffer (PBS) was prepared by adding 10.72 mM sodium phosphate dibasic and 19.28 mM sodium phosphate monobasic to purified water. The osmolality was adjusted to 270 mOsm/kg by adding sodium chloride, and the pH was adjusted to 6.50 with hydrochloric acid or sodium hydroxide. Biomimetic media were prepared at varying bile salt (sodium taurocholate) and phospholipid (lecithin) concentrations corresponding to 0.17 times up to 5 times that found in FaSSIF_V1 by adding 0.38 − 11.2 g/L of FaSSIF/FeSSIF/

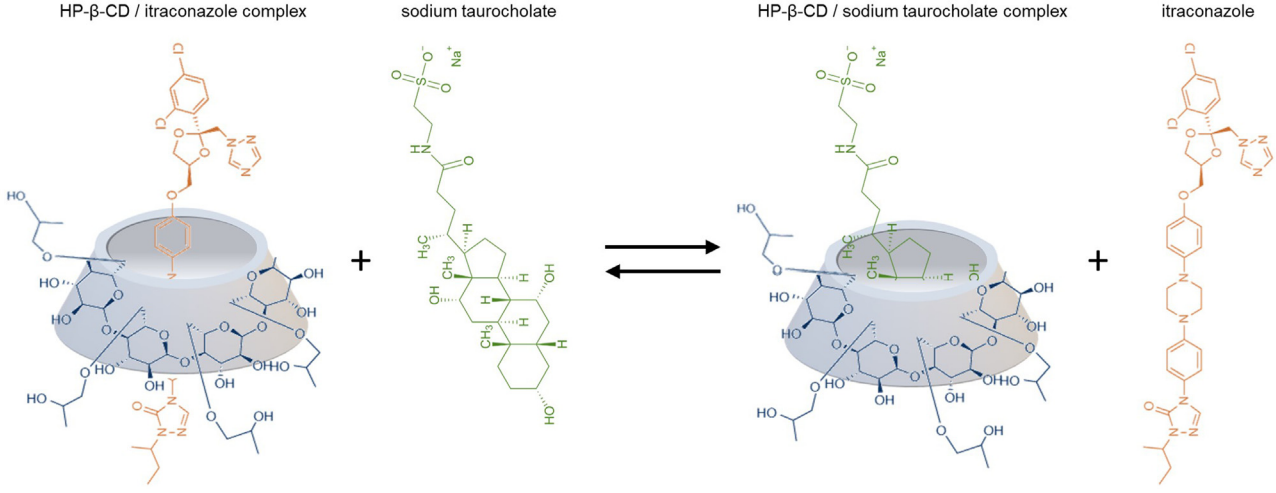

Figure 1. Schematic illustration of sodium taurocholate displacing itraconazole from the hydrophobic cavity of HP-β-CD.

FaSSGF powder to the PBS. Media for the solubility test had varying concentration of HP-β-CD added. Double concentrated buffer, which was used in the donor for permeability studies to dilute the gastric media solution with Sporanox® Oral Solution, was prepared by adding double the amount of all the components to the buffers, NaOH was added upon mixing of the double concentrated buffer with gastric media to achieve pH 6.5.

The gastric media (FaSSGF) was prepared by adjusting the pH of purified water to 2 and the osmolality to 270 mOsm/kg by adding sodium chloride.

The acceptor medium for permeation experiments was prepared by dissolving 2-HP-β-CD in a hydrochloric acid solution (pH 1.5, 270 mOsm/kg) for a final concentration of 64.8 mM. The osmolality was adjusted to 508 mOsm,/kg, equivalent to that of the donor media upon the addition of Sporanox® Oral Solution.

Phase Solubility Studies

The solubility of itraconazole was investigated in the 30 mM PBS containing HP-β-CD (0 / 5.2 / 13.0 / 25.9/ 51.9 mM) or FaSSIF_V1 (0x, 0.17x, 0.5x, 1x, 2.7x and 5x; the description refers to the relative concentration of sodium taurocholate and lecithin compared to the original FaSSIF_V1 protocol with 3 mM sodium taurocholate and 0.75 mM lecithin[19]). Moreover, combinations of FaSSIF_V1 and 5x FaSSIF_V1 (simulating the fed state in the small intestine with respect to bile salt and lecithin concentrations) with the 5 mentioned HP-β-CD concentrations were also tested. For the test, 40 mg of itraconazole were added to 20 mL of each medium for a final concentration of 2 mg/mL, and after being vortexed for 10 s, the samples were subjected to constant stirring at 150 rpm and 37 °C for 24–48 h in a heated water bath. After the incubation, three samples of 1 mL each were taken from each vial and centrifuged at 14,000 rcf and 37 °C for 20 min. The supernatant of each sample was filtered through Whatman® Anotop® syringe filters with a pore size of 0.2 μm (GE Healthcare, Buckinghamshire, UK) and diluted with acetonitrile. The prepared samples were finally analyzed with reverse-phase HPLC-UV.

Permeation Screening Studies in the PermeaPad® Plate

A preliminary screening of Sporanox® Oral Solution dissolution/permeation was performed using PermeaPad® 96-well plates.[20] In the acceptor compartment (200 μL top chamber), a 64.8 mM HP-β-CD solution at pH 1.5 was used in all cases. In the donor compartment (300 μL bottom chamber), at the start of the experiments 150 μL double concentrated 0x, 0.17x, 0.5x, 1x, 2.7x or 5x FaSSIF_V1 were mixed with 1:1 with 150 μL of Sporanox® Oral Solution in FaSSGF to obtain a final concentration of itraconazole at 2.8 mM and 51.9 mM HP-β-CD (corresponding to the maximum HP-β-CD concentration obtained *in vivo* in human duodenal sample after the administration of Sporanox® Oral Solution without water[21]). It should be noted that the Sporanox® Oral Solution was incubated in FaSSGF for 30 min before the experiment, and that NaOH was added upon the mixture in the donor to maintain pH 6.5. During the experiment, the system was kept in an oven at 37 °C, and each donor cell was stirred. The permeated drug amount was quantified in the acceptor after 240 min; samples were diluted 1:1 with acetonitrile before HPLC-UV analysis.

Dissolution/Permeation Studies in Side-by-Side cells

Itraconazole's dissolution/permeation was assessed in side-by-side cells. The system had a 5 mL acceptor and 7 mL donor compartment separated by the PermeaPad® barrier. The compartments were stirred at 500 rpm with stir bars and heated to 37 °C by a circulating water system. Like in the 96-well plate experiments, the acceptor compartment was filled with a 64.8 mM HP-β-CD solution at pH 1.5 while the donor compartment contained a 1:1 mixture of double concentrated 0x, 0.17x, 0.5x, 1x, 2.7x or 5x FaSSIF_V1 and Sporanox® Oral Solution in FaSSGF to obtain a final concentration of 2.8 mM itraconazole and 51.9 mM HP-β-CD. It should be noted that the Sporanox® Oral Solution was incubated in FaSSGF for 30 min before the experiment, and that NaOH was added to the mixture in the donor to maintain pH 6.5.

Samples of 50 μL each were withdrawn from the donor compartments after 5, 15, 30, 60, 120, 180 and 240 min. The acceptor samples, of the same volume, were taken at 5, 60, 90, 120, 180 and 240 min. Media were not replaced in either compartment. Samples from the donor cells were centrifuged at 14,000 rcf for 5 min, and the supernatant was diluted 1:3 in acetonitrile. Meanwhile, samples from the acceptor cells were directly diluted 1:1 in acetonitrile. All samples were analyzed with HPLC-UV and the data were corrected taking into consideration the volume withdrawn with each sample.

Quantification by HPLC-UV

Itraconazole was quantified using reverse-phase HPLC-UV method with a C18 stationary phase (Acclaim® 120 C18 column; 4.6 × 150 mm, 3 μm) heated to 50 °C during runs. The measurements were performed at 260 nm. The mobile phase had a flow rate of 1.0 mL/min and consisted of 33.25% (v/v) of 5 mM ammonium acetate solution pH 9.3 and 66.75% (v/v) acetonitrile. The limit of detection and quantification were determined to be 14.67 ng/mL and 44.47 ng/mL, respectively.

Results

Phase Solubility Studies

The data reported in Fig. 2A show the (apparent) equilibrium solubility of itraconazole in the absence and presence of different concentrations of HP-β-CD (no bile salts). The results are in accordance with the literature data. They indicate that HP-β-CD strongly enhances itraconazole solubility (from 0.07 μM with no HP-β-CD to 47 μM with 51.9 mM HP-β-CD) even though the correlation is not linear. Brewster et al., 2008, suggested that the correlation between HP-β-CD and itraconazole can be described as an A_p-type profile, which indicates the formation of higher order complexes at higher HP-β-CD concentrations.[22]

Fig. 2B illustrates how increasing concentrations of bile salts (from FaSSIF/FeSSIF/FaSSGF powder) influence itraconazole solubility (no HP-β-CD present). The concentrations of bile salts cover a wide range of values obtained from human samples from the lowest ones in fasted state up to average concentration of the fed state. In the absence of HP-β-CD, the solubility of itraconazole increases with the increment of bile salts, from 0.07 μM with no bile salts present to 6.7 μM when in presence of 15 mM of taurocholate and 3.75 mM lecithin (5x FaSSIF_V1). The apparent solubility of the drug is proportional to the FaSSIF_V1 concentration.

In Fig. 3A the solubilities of itraconazole in absence and presence of bile salts and phospholipids (low concentration as in FaSSIF_V1 and a high concentration as in 5x FaSSIF_V1, corresponding to the sodium taurocholate and lecithin concentrations in fed state simulated intestinal fluid (FeSSIF_V1), are presented with respect of different concentrations of HP-β-CD. In all cases, HP-β-CD strongly increases itraconazole solubility but, in comparison to the absence of bile salts, itraconazole solubility in both the fasted and 5x FaSSIF state resulted in an impaired solubility increase from HP-β-CD compared to the PBS. Moreover, the above-mentioned non-linear relationship between the solubility and HP-β-CD concentration is found in all cases. Nonetheless, for the lowest HP-β-CD concentrations (0 and 5.2 mM) itraconazole solubility is improved by the presence of bile

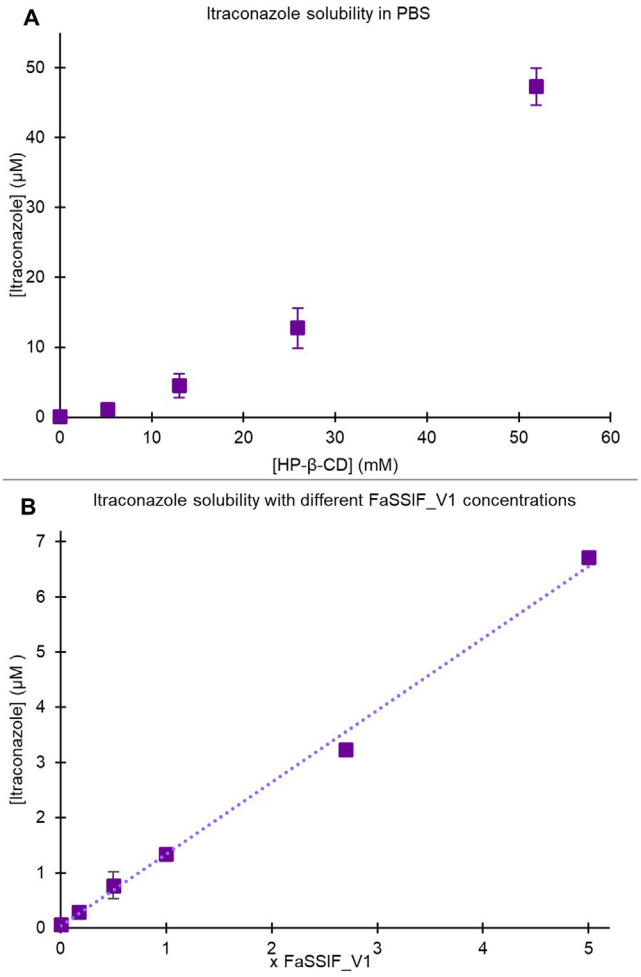

Figure 2. (A) Itraconazole solubility in PBS with different concentrations of HP-β-CD. All data are reported as mean ± SD (n = 3). (B) Itraconazole solubility in presence of different concentrations of bile salts obtained using the FaSSIF/FeSSIF/FaSSGF powder. All data are reported as mean ± SD (n = 3).

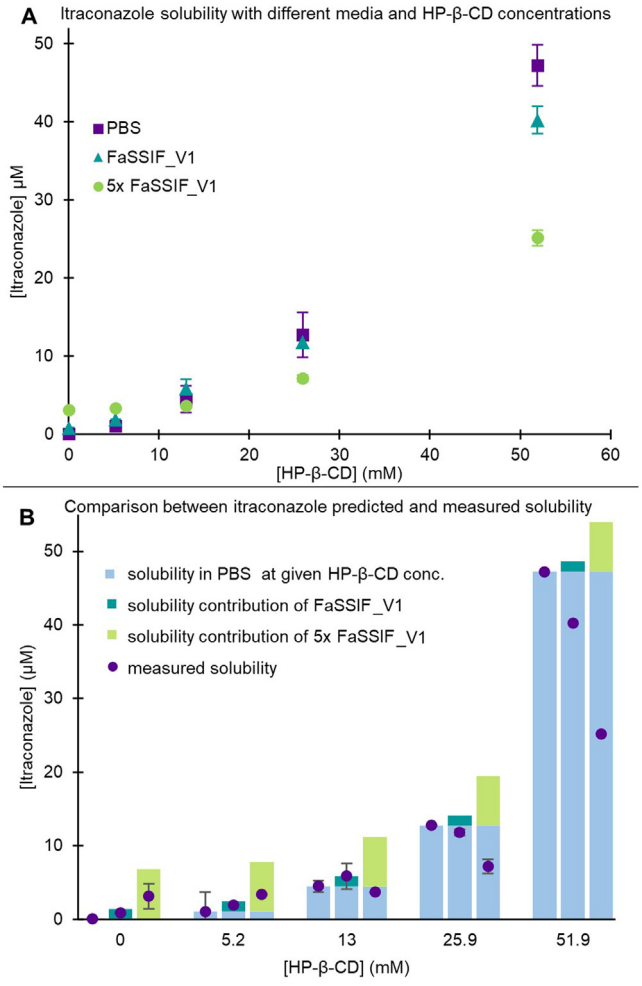

Figure 3. (A) Itraconazole solubility in FaSSIF and 5xFaSSIF (fasted and fed states) and different concentrations of HP-β-CD. All data are reported as mean ± SD (n = 3). (B) Comparison between the predicted solubility of itraconazole in different media and the measured solubility in those media, in presence of different concentrations of HP-β-CD. The bars represent the sum of itraconazole itraconazole solubility in PBS and in FaSSIF and 5x FaSSIF (average values reported in Fig. 1A and B). The dots represent the measured solubility in those media as mean ± SD (n = 3).

salts. However, solubility is negatively influenced by the presence of bile salts for the highest HP-β-CD concentrations (25.9 and 51.9 mM). With higher concentrations of bile salts, this effect is more evident, *e.g.* when the taurocholate/lecithin concentration from the fed state is mimicked (5x FaSSIF_V1) in comparison to the fasted state (FaSSIF_V1).

Fig. 3B compares predicted and experimentally obtained solubility values of itraconazole in different media. Predictions were made as the sum of the solubility in PBS in presence of different HP-β-CD concentrations plus those obtained in FaSSIF_V1 or 5x FaSSIF_V1 (without cyclodextrin), assuming no interactions between HP-β-CD and the FaSSIF_V1. The results reported in Fig. 3A and 3B show an increase of predicted solubility with increasing amounts of bile salts present. However, only for conditions for low HP-β-CD concentrations (0 and 5.2 mM HP-β-CD) the prediction is within reasonable limit, while for higher HP-β-CD concentrations the measured solubility deviates largely due to the presence of bile salts/phospholipids.

Permeation Studies

The results from itraconazole dissolution/permeation screening in PermeaPad® 96-well plates are reported in Fig. 4A. The results for 240 min of permeation of the drug show that the presence of bile salts reduces permeation: decreasing from 0.29 μg permeated drug when none was present to 0.04 μg with the highest bile salt concentration, mimicking the bile salt/phospholipid concentration in the fed state with 5x FaSSIF_V1.

Fig. 4B correlates the obtained results of itraconazole apparent solubility and permeability (reported in Figs. 2A and 3A) in PBS, FaSSIF_V1 and 5x FaSSIF_V1. As it is shown in the graph, the ranking is the same. However, no direct proportional correlation was found.

Experiments in side-by-side cells were carried out to further investigate the dissolution/permeation of itraconazole in the presence of both bile salts and HP-β-CD. The results describing the apparently dissolved itraconazole in the donor and itraconazole permeating into the acceptor cells are reported in Fig. 5, respectively A and B.

In the donor cells, when no bile salts were present and after the gastric step, the concentration of itraconazole in solution remained constant and stable in a supersaturated state throughout the permeation experiment. On the other hand, intense precipitation was observed in the presence of bile salts; the higher the concentration of bile salts, the more pronounced the precipitation. Moreover, the increasing bile salt concentrations lowered the initial degree of supersaturation. At lower bile salt concentrations (0.17x and 0.5x FaSSIF_V1), the initial itraconazole concentrations were closer to the one obtained with PBS. The precipitation of the drug took longer:

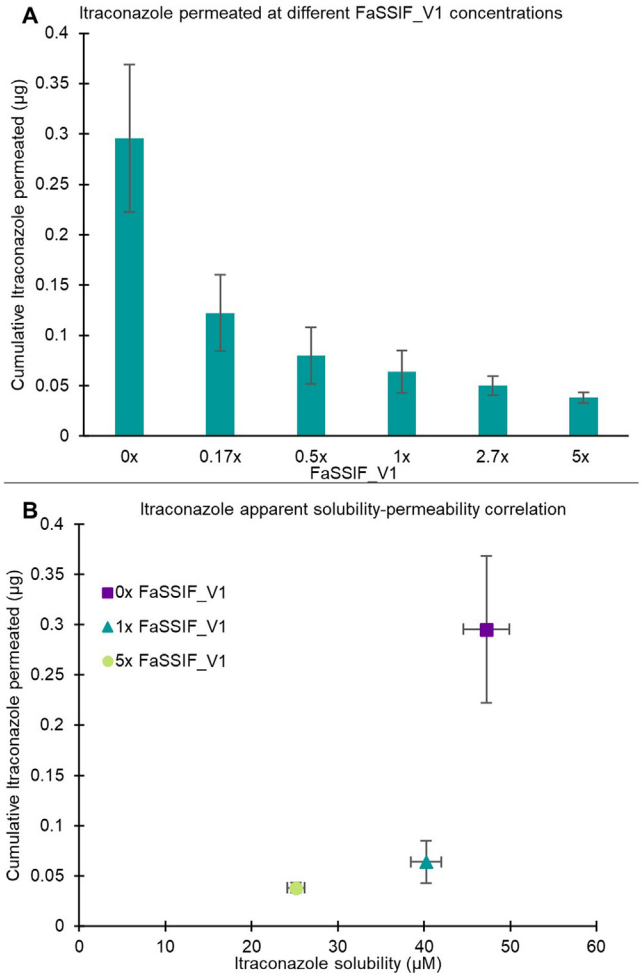

Figure 4. (A) screening assessing the permeation of HP-β-CD-formulated itraconazole when in presence of different concentrations of bile salts after 4 h. All data are reported as mean ± SD (n = 5). (B) correlation between itraconazole apparent solubility and permeability in different FaSSIF concentrations (0x, 1x and 5x). Both solubility and permeability values are reported as mean ± SD (n = 3).

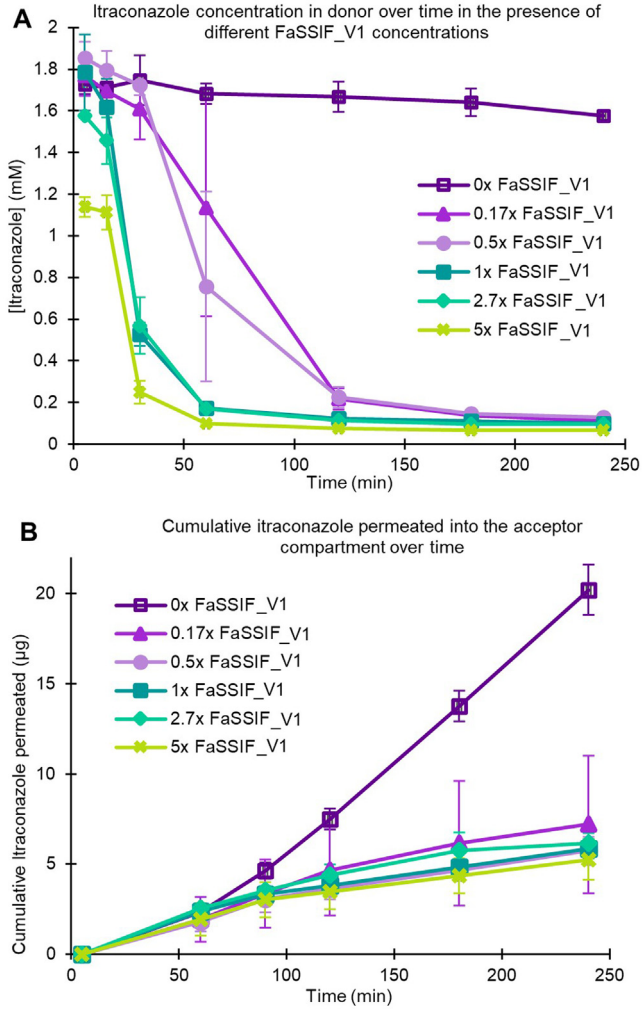

Figure 5. (A) HP-β-CD-formulated itraconazole concentrations over time in donor cells when in presence of different concentrations of bile salts. All data are reported as mean ± SD (n = 3). (B) cunulative permeated amount of itraconazole in the acceptor cells over time. Acceptor media composition: 10% w/v HP-β-CD, pH=1.5. All data are reported as mean ± SD (n = 3).

itraconazole strongly precipitated during the first two hours, but without reaching the low concentration values obtained in the case of the higher bile salts concentrations until 3 h after the beginning of the experiment. However, highest bile salts concentrations (5x FaSSIF_V1), caused the initial concentration of itraconazole to be significantly reduced, in proportion to the increased taurocholate concentration, and the precipitation occurred within the first hour. Nonetheless, in all conditions and at all time points, the concentration of itraconazole remained higher than the solubility obtained in the respective phase solubility study.

In Fig. 5B, the amount of itraconazole permeated across the PermeaPad® barrier over time for every donor condition previously described is reported. The strongest permeation increment was obtained when no bile salt was present in the corresponding donor compartments, which correlates with the more stable solution observed in the donor cells. In the presence of bile salts, the permeation was strongly impaired and the increase of itraconazole in the acceptor cells was minimal. Specifically, when no bile salts were present in the donor, the cumulated amount of permeated itraconazole increased over time, reaching 20.2 μg after 4 h; meanwhile, increasing the bile salt concentration in the donor, the permeation was strongly reduced, ranging between 7.2 and 5.2 μg after 4 h (2.8 to 3.9 fold reduction in comparison to no-bile salts in the donor cells).

Moreover, unlike the results obtained with the permeation screening on the microtiter plates, the rank order of drug permeated amount did not fully correlate with the increasing order of bile salts concentrations. This is probably due to the lower number of replicates and a certain degree of randomness in the precipitation from supersaturated solutions.

Discussion

The phase solubility study in the presence of increasing concentrations of HP-β-CD (no bile salts) is in good agreement with data from the literature showing a non-linear correlation between apparent solubility of itraconazole and HP-β-CD concentrations, suggesting the formation of higher-order complexes between the drug and the formulation.[23] Moreover, it was possible to appreciate differences between different media. These data suggest that when no or low concentrations of HP-β-CD are present, the presence of bile salts helps to keep itraconazole in solution forming micelles that can incorporate the drug molecules and prevent precipitation. However, for higher HP-β-CD concentrations, when HP-β-CD becomes the main factor interacting with the drug and improving its solubility,

the presence of bile salts has a negative effect, which is more evident for higher bile salt concentrations.

This phenomenon can be explained by the competition between bile salts and itraconazole to fit inside the molecular cavity of HP-β-CD, likely displacing the itraconazole molecules and thus inducing the spontaneous self-assembly of itraconazole molecules in the solution to form crystals and precipitation. The tendency for itraconazole to precipitate in the presence of HP-β-CD and FaSSIF_V1 had already been observed by in a former study.[24]

For the dissolution/permeation studies, the range of HP-β-CD concentrations taken into account was based on the maximum HP-β-CD concentrations obtained from samples taken *in vivo* from human duodenum after the oral administration of Sporanox®.[21] In contrast, the considered bile salt concentrations represent a rough assumption of the human *in vivo* bile salt concentration range based on human samples in the fasted state made by Riethorst and co-workers.[6] This experimental design allows to evaluate how the dissolution/permeation interplay of Sporanox® connects to individual variability in humans.

The screening studies on the PermeaPad® 96 well plate revealed a clear trend: the total amount of itraconazole permeated decreased with higher concentrations of bile salts (and phospholipids) in the media. The decrease was particularly pronounced when comparing PBS (no bile salts or phospholipids) and low concentrations of FaSSIF_V1. Such results suggest a correlation between the individual variability in bile salt concentrations and the bioavailability of itraconazole, which could justify the variability in the bioavailability data obtained *in vivo* by Berben et al..[21] Moreover, these results are in contrast to our previous observations with Albendazole,[14] where we observed that bile salts push the albendazole out of the HP-β-CD complex, increase the amount of molecularly dissolved drug compound and increase the permeation. However, the permeation of albendazole was studied for solutions/suspensions in equilibrium, while in the present study, the pH shift and dilution of Sporanox® caused itraconazole to be present in an unstable supersaturated state.

The experiments in side-by-side cells gave insight into the processes in the donor compartment. Here, we observed that precipitation did not occur if no FaSSIF_V1 was present. However, as soon as just a small amount of FaSSIF_V1 was added, the Sporanox® started to precipitate within 30 min. We observed that more concentrated FaSSIF_V1 seems to cause faster precipitation. However, due to few replicates and expected randomness in precipitation processes, the ranking was not as good as in the 96-well plate experiment.[25] The results from this study indicate that HP-β-CD in Sporanox® acts as a stabilizer of the supersaturation caused by the pH shift upon the transfer from gastric to intestinal conditions. This stabilizing effect of HP-β-CD is impaired by the bile salts (and phospholipids) in biomimetic media, even at low concentrations, which would only decrease the apparent solubility to a very moderate extent.

Stappaerts and co-workers performed gastric transfer studies with itraconazole in the presence of bile salts and HP-β-CD.[24] In their study, the stability of the supersaturation in the absence of FaSSIF_V1 was more similar to the stability of the supersaturation in the presence of FaSSIF_V1. However, in contrast to our study, they used concentrations reflecting 75–80% saturated solutions of itraconazole drug compound at the gastric step, while we used the commercial drug product Sporanox®, which contains a high amount of propylene glycol, which in previous studies with other compounds has shown precipitation-inhibitory effect and no effect on supersaturation.[26,27] We assume that differences in dose/volume and the presence of propylene glycol are the reason for the observed higher degrees of supersaturation upon dilution; an alternative hypothesis may be that our study included an absorptive sink compartment. Previous studies have shown that supersaturation in the presence of an absorptive compartment tends to change the dissolution/precipitation kinetics.[28]

The finding of less drug permeation with higher FaSSIF_V1 concentrations agrees with the slight negative *in vivo* food effect of Sporanox® Oral Solution (fed/fasted AUC ratio 0.77).[29] Negative food effect due to less drug permeation across the intestinal barrier via passive diffusion is a rare case for poorly soluble drug compounds.[30] However, it makes sense for a cyclodextrin formulation as the bile salts and phospholipids will work against keeping the itraconazole dissolved by the complex rather than helping it to stay in solution in micelles and mixed micelles as usual otherwise. This is also the reason why the capsule formulation, that does not contain cyclodextrins, shows the opposite, namely a positive food effect *in vivo*.[17] In a recent study[18] with the Tiny-TIM system, it was found that this integrated set-up predicted a positive food effect of the Sporanox® oral solution, which is proven wrong *in vivo*. One of the obvious differences between the two set-ups is that Tiny-TIM contains a polysulfone plasma filter with a pore size of $\leq$ 50 nm,[31] which is not biomimetic and sorts particles after their sizes, while we used a biomimetic barrier which can discriminate species according to their transition abilities through a swollen vesicular phospholipid layer.[20,32]

Conclusion

We investigated the effect of biomimetic media of different bile-salt concentrations on Sporanox® Oral Solution in terms of dissolution and permeation. Two-stage in-vitro dissolution/permeation studies indicate that the itraconazole in Sporanox® Oral Solution, when administered orally, as a consequence of the pH change from the acidic gastric environment to the more neutral intestinal pH and/or the dilution during ingestion, will transiently reach dissolved concentrations above its solubility limit, *i.e.*, supersaturation. Interestingly, this supersaturation is highly sensitive to bile salt concentrations: it is relatively stable in plain buffer, *i.e.*, when no bile salts or phospholipids are present. However, even at low concentrations, bile salts competitively squeeze itraconazole out of the cyclodextrin complex, causing an even higher degree of supersaturation, and itraconazole starts crushing out within 30 min. Therefore, the permeation was also found to be reduced. This observation made by rather simple dissolution/permeation *in vitro* tools is in good agreement with the negative food effect reported *in vivo*, an effect that another *in vitro* tool (Tiny-TIM) had failed to predict.

Declaration of Competing Interest

Annette Bauer-Brandl is a co-inventor of PermeaPad® (European Patent EP3221687, owned by University of Southern Denmark). No conflict of interest to declare.

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