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Gastrointestinal behavior of itraconazole in humans – Part 2: The effect of intraluminal dilution on the performance of a cyclodextrin-based solution

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ABSTRACT

Hydroxypropyl-β-cyclodextrin (HP-β-CD) is known to enable absorption of the lipophilic drug itraconazole. Since the interaction between HP-β-CD and itraconazole is characterized by a non-lineair, A_P-type phase-solubility diagram, the present study aimed to investigate the influence of intraluminal dilution (water intake) on the behavior and performance of an orally administered cyclodextrin-based solution of itraconazole. Subsequently, the *in vivo* behavior was simulated by combining *in vitro* dilution with permeation assessment. After the administration of a Sporanox® solution to healthy volunteers with or without a glass of water, gastrointestinal and systemic concentrations of itraconazole were simultaneously monitored. Independently of the intake of water, no gastric precipitation of itraconazole was observed. After transfer to the duodenum, precipitation occurred and was more pronounced in the condition with water, resulting in a 7.6-fold reduction in duodenal $AUC_{0\text{-}3h}$ compared to the condition without water. Nevertheless, plasma concentration-time profiles did not demonstrate any significant differences in $AUC_{0\text{-}8h}$, C_{max} and t_{max}. Application of freshly aspirated intestinal fluids on Caco-2 cells clearly confirmed that higher intestinal itraconazole concentrations after intake of Sporanox® without water do not generate a substantially increased itraconazole uptake. A two-stage *in vitro* dilution test was combined with a permeation compartment to capture this solubility-permeability interplay. In conclusion, this work demonstrates that variations in intraluminal dilution may have a drastic impact on the gastrointestinal behavior of lipophilic drugs in the presence of cyclodextrins. In the case of an AP-type interaction with cyclodextrins, the trade-off between solubility and permeability may be affected.

1. Introduction

The fraction of a drug reaching the blood circulation after oral administration typically depends on both the drug concentration in intestinal fluids and the permeability of the intestinal epithelium (Buckley et al., 2012). In current drug development programs, cyclodextrins are widely used as pharmaceutical excipients to improve the oral bioavailability of poorly water soluble compounds by enhancing the solubility in the formulation and/or in aqueous intraluminal fluids. Since up to 70% of the new chemical entities (NCE) are suffering from poor water solubility according to the Biopharmaceutical Classification System (BCS), cyclodextrins are gaining reputation from formulation scientists (Amidon et al., 1995; Dahan and Hoffman, 2008; Davis and Brewster, 2004; Faller and Ertl, 2007; Kawabata et al., 2011; Lipinski, 2000; Loftsson and Brewster, 1996; Moos et al., 1993; Singh et al., 2011; Vieth et al., 2004). From a structural point of view, cyclodextrins are cyclic oligosaccharides creating an interior hydrophobic cavity with exterior hydrophilic properties (Brewster and Loftsson, 2007; Loftsson and Brewster, 1996; Rajewski and Stella, 1996). Due to this design, lipophilic compounds can be captured by the hydrophobic core, whereas the hydrophilic exterior will interact with the surrounding water. As such, these so-called inclusion complexes enable increased intraluminal concentrations of poorly water soluble drugs. A number of *in vitro* studies, however, indicated that these increased intraluminal concentrations may not necessarily imply enhanced absorption, since inclusion in cyclodextrins may restrict the fraction of the drug readily available for permeation through the intestinal epithelium. This dual effect is called the solubility-permeability

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interplay (Beig et al., 2015, 2013b, 2013a; Dahan et al., 2010; Miller and Dahan, 2012). This phenomenon has also recently been demonstrated in rats whereby an increasing concentration of hydroxypropyl-β-cyclodextrin (HP-β-CD) reduced the bioavailability of danazol, which has a low aqueous solubility and high affinity for HP-β-CD. In contrast, the reduced bioavailability at increasing concentrations of HP-β-CD was not observed for cinnarizine, which has both a lower affinity for HP-β-CD and a higher aqueous solubility (Holm et al., 2016).

A well-known example of the use of cyclodextrins to improve oral bioavailability, is a cyclodextrin-based oral solution of the lipophilic (clogP 6.2) and weakly basic (pKa 2 and 3.7) drug itraconazole. In this formulation (Sporanox® solution, Janssen, Beerse, Belgium), itraconazole (aqueous solubility approximately 1 ng/ml) is solubilized by means of 40% 2-hydroxypropyl-β-cyclodextrin (Brewster and Loftsson, 2007; Peeters et al., 2002; Six et al., 2005). The relationship between itraconazole solubility and HP-β-CD concentrations demonstrates a non-linear dependence (A_p-type profile), indicating that higher order complexes are formed at increasing cyclodextrin concentrations (Brewster et al., 2008). As such, it can be hypothesized that cyclodextrin effects on the solubility and permeability of itraconazole may be influenced by the extent of intraluminal dilution and thus by the amount of water co-administered with the Sporanox® solution. Typically, the bulk of medicines are administered with a standardized volume of water (240 ml) in clinical trials and bio-equivalence studies, according to the FDA guidelines ("Food-Effect Bioavailability and Fed Bioequivalence Studies – UCM126833.pdf," n.d.). However, the patient leaflet of the Sporanox® solution recommends oral administration without any water intake. Nevertheless, in daily practice, patients often dose their medication with an unstandardized volume of water (or even another beverage) (Rubbens et al., 2016), implying that real-life dosing conditions may cause variable cyclodextrin effects on intraluminal drug behavior and intestinal absorption.

The present study aimed to investigate the impact of water intake, and thus intraluminal dilution, on the gastrointestinal behavior and performance of the cyclodextrin-based itraconazole solution. To this end, the Sporanox® solution (20 ml) was orally administered to healthy volunteers (A) with and (B) without a standardized volume of water (240 ml). The influence of dilution on both intraluminal and systemic itraconazole concentrations was assessed by simultaneously collecting gastrointestinal and plasma samples during 3 and 8 h, respectively. To capture different dilution ratio effects on not only the intraluminal concentration of itraconazole but also on its permeation across the intestinal epithelium, human intestinal fluids freshly aspirated after intake of the Sporanox® solution (with or without water), were instantaneously applied as the donor solution in a Caco-2 assay. Since there is a high need for the development of biopharmaceutical models predictive for oral formulation performance (Kostewicz et al., 2014), our collected *in vivo* data were subsequently used as reference for the evaluation of a simple *in vitro* gastrointestinal transfer assay in combination with a Caco-2 cell monolayer as a predictive tool for the performance of cyclodextrin-based solutions.

2. Materials & methods

2.1. Chemicals

Itraconazole and hydroxy-itraconazole were obtained from Johnson & Johnson (Beerse, Belgium). Deuterated itraconazole ($^2H_5$-itraconazole) and deuterated hydroxy-itraconazole ($^2H_6$-OH-itraconazole) were purchased from Alsachim (Illkirch, France). Dimethyl sulfoxide (DMSO), methanol (MeOH), glucose and type III porcine mucin were provided by Acros Organics (Geel, Belgium). Sodium acetate trihydrate, acetic acid and sodium chloride (NaCl) were purchased from VWR (Leuven, Belgium), while sodium hydroxide (NaOH) pellets were purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN) was supplied by Fisher Scientific (Leicestershire, UK) and sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$) was supplied by Sigma Aldrich (St. Louis, MO, USA). Hanks' Balanced Salt Solution without phenol red (HBSS), Dulbecco's Modified Eagle's Medium (DMEM), 4-(2-hydroxyetheyl)-1-piperazineethanesulfonic acid (HEPES), 10.000 IU/ml penicillin, 10.000 μg/ml streptomycin, nonessential amino acid medium (NEAA), trypsin ethylenediaminetetraacetic acid (EDTA) and fetal bovine serum (FBS) were obtained from Lonza (Verviers, Belgium). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was supplied by Eastman Chemical Company (Kingsport, TN). FaSSIF/FeSSIF/FaSSGF powder was purchased from Biorelevant (Croydon, UK). Water was purified using a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

2.2. Media

Transport medium ($HBSS^+$) was prepared by dissolving 25 mM glucose in 500 ml HBSS, buffered with 10 mM HEPES and adjusted to pH 7.4 with 2 M of NaOH. Caco-2 cell culture medium consisted of DMEM supplemented with 10% FBS, 1% nonessential amino acids, 100 IU/ml penicillin and 100 μg/ml streptomycin ($DMEM^+$). A mucus layer, serving as a protective layer for the cells consisted of type III mucin derived from porcine stomach dissolved in $HBSS^+$ (50 mg/ml) and adjusted to pH 6.5. Fasted state simulated gastric fluid (FaSSGF) and fasted state simulated intestinal fluid (FaSSIF) were prepared according to standardized protocols supplied by Biorelevant. To obtain FaSSGF, FaSSIF/FeSSIF/FaSSGF powder (0.060 mg/ml) was added to blank FaSSGF (1.999 g NaCl dissolved in 1 l purified water, adjusted to pH 1.6 with 2 M HCl). A higher amount of the same powder (2.24 mg/ml) was dissolved in blank FaSSIF (3.95 mg/ml $NaH_2PO_4 \cdot H_2O$, 6.19 mg/ml NaCl, 0.42 mg/ml NaOH, adjusted to pH 6.5) to end up with FaSSIF (3 mM taurocholate, 0.75 mM lecithin). To prepare double-concentrated FaSSIF (2x FaSSIF), double amounts of $NaH_2PO_4 \cdot H_2O$, NaCl, NaOH and FaSSIF/FeSSIF/FaSSGF powder were dissolved in purified water and adjusted to pH 7.4.

2.3. Caco-2 cell culture

Caco-2 cells were cultured in cell culture flasks at 37 °C in an atmosphere of 5% CO_2 and 90% relative humidity in $DMEM^+$. Every 3-4 days, cells were passaged at 80–90% confluence using trypsin-EDTA. For transport experiments, Caco-2 cells were seeded on porous Costar Transwell membrane inserts (Polycarbonate 3 μm pore diameter, 12 mm diameter, 1.13 cm^2 growth area, Corning Inc., Corning, NY) at a density of 90.000 cells/cm^2. Cells (passage number 67-96) were used for transport experiments between 17 and 21 days after seeding.

2.4. In vivo *studies in healthy volunteers*

2.4.1. In vivo study design

To evaluate the effect of concomitant water intake on the behavior of the cyclodextrin-based Sporanox® solution, a cross-over study (2 conditions: with and without water) was performed at the University Hospitals Leuven in 5 healthy human volunteers (HV) (3 males and 2 females aged between 22 and 27 years old). Volunteers could only participate in the study after a short medical examination. Participation of volunteers suffering from gastrointestinal disorders or infection diseases (hepatitis B or C and HIV) was strictly forbidden, protecting the well-being of the researchers and volunteers. Other exclusion criteria were the regular intake of medication, frequent exposure to X-rays or a potential pregnancy.