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ScienceDirect
International Journal of Pharmaceutics 357 (2008) 169–179
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Ordered mesoporous silica induces pH-independent supersaturation of the basic low solubility compound itraconazole resulting in enhanced transepithelial transport

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Received 29 December 2007; received in revised form 22 January 2008; accepted 25 January 2008
Available online 3 February 2008

Abstract

The majority of innovative drug candidates are poorly water soluble and exhibit basic properties. This makes them highly dependent on the *in vivo* encountered acid–neutral pH sequence to achieve a sufficient dissolution and thus absorption. In this study, we evaluated the pH-independent generation of intraluminally induced supersaturation of the model compound itraconazole and its beneficial effect on the extent of absorption in the Caco-2 system and the rat *in situ* perfusion system. Local supersaturation was obtained by means of a solvent shift method and a novel formulation strategy based on ordered mesoporous silica (OMS) as a carrier. *In vitro* results evidenced that both methods were capable of creating a supersaturated state of itraconazole in fasted state simulated intestinal fluid (FaSSIF) when no preceding acidic dissolution was simulated. The extent of supersaturation exceeded 21.9 and 9.6 during at least 4 h for the solvent shift method and OMS as a carrier, respectively. As compared to saturation conditions (0.09 ± 0.01 μg), supersaturation induced by the solvent shift method as well as by the use of OMS increased transport across a Caco-2 cell monolayer more than 16-fold, resulting in the basolateral appearance of 2.20 ± 0.29 μg and 1.46 ± 0.03 μg itraconazole after 90 min, respectively. In the absence of an acid–neutral pH sequence, the performance of the marketed product Sporanox® was inferior with total transport amounting to 0.12 ± 0.03 μg after 90 min. Enhanced absorption was confirmed in the *in situ* perfusion model where OMS was able to boost total transport of itraconazole after 60 min from 0.03 ± 0.01 nmol cm^{-1} to 0.70 ± 0.09 nmol cm^{-1} compared to saturated equilibrium conditions in FaSSIF. The solid dosage form Sporanox® again failed to achieve a similar extent of absorption enhancement (0.29 ± 0.01 nmol cm^{-1}). These findings suggest that intraluminal supersaturation can be created by the use of OMS and that preceding dissolution of basic compounds in the acidic medium of the stomach is not required to allow for efficient intestinal absorption. The use of OMS appears to be a promising strategy for the delivery of especially basic low solubility compounds in patients suffering from hypochlorhydria; the pH independency may also result in a more reproducible systemic exposure.

Keywords: Supersaturation; Drug absorption; Ordered mesoporous silica; Itraconazole

1. Introduction

The majority of today's pharmaceutical compounds are poorly water soluble. Also the new bioactive organic compounds that result from high-throughput screening programs tend to be very little soluble in water (Lipinski, 2000). Such compounds may suffer from insufficient dissolution throughout the gastrointestinal tract and therefore achieve inferior systemic exposure after oral administration. Today, failing bioavailability is one of the main reasons for abandoning innovative oral drug candidates. Circumventing low solubility and unfavourable dissolution equilibrium kinetics are the key issues in the development of an appropriate formulation (Allen and Cullis, 2004).

An attractive strategy to enhance oral bioavailability is the use of a formulation, which creates supersaturation in the gastrointestinal environment. If drug concentrations are maintained

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doi:10.1016/j.ijpharm.2008.01.049

many times higher than the thermodynamic solubility during a sufficient time period, enhanced absorption can be expected (Hou and Siegel, 2006). The concept of supersaturation has been probed by various research groups, mainly focussing on the development of topical drug delivery systems to enhance drug permeation (Pellett et al., 1997). Accompanied with this absorption improvement, there is a reduced need to incorporate large amounts of solubility enhancing excipients which may result in a lower toxicity profile of the vehicle.

In the case of drugs that are (weak) bases, the ability to create supersaturated solutions depends on gastric acidity (Kostewicz et al., 2002). Such an acidic environment enables enhanced dissolution of basic compounds through protonation and avoids crystal precipitation due to electrostatic repulsion and higher intrinsic solubility in acidic media. Following a pH shift upon arrival in the small intestine (neutral pH), the maintenance of a supersaturated state could allow an increased absorption of the poorly water-soluble compound (Gu et al., 2005). Formulation specialists often speculate on this gastro-intestinal pH sequence to foster the oral bioavailability of poorly water-soluble compounds by using solid dispersions or self-emulsifying systems that allow rapid dissolution at acidic pH of the stomach (Guzmán et al., 2004; Gao et al., 2004; Gao and Morozowich, 2006). An example of this methodology is the capsule-based dosage form Sporanox® which contains itraconazole as an active ingredient. In this formulation, dissolution of the water-soluble HPMC phase is associated with the release of itraconazole at concentrations above its saturation solubility in the gastric medium. Some authors have speculated that the microenvironment created by this relatively slow dissolving HPMC protects the drug from precipitation when leaving the stomach (Six et al., 2005). Supersaturated concentrations are presumed to be maintained after the sharp decrease of equilibrium solubility in the small intestine long enough to result in an increased oral bioavailability (Brewster et al., 2004).

In case of physiological malfunctions associated with hypochlorhydria, however, the condition of an initial acidic environment to dissolve basic drugs is not fulfilled. For instance, HIV-infected patients often suffer from a decreased acidity in the stomach (Welage et al., 1995); therefore significant effects on the oral bioavailability of poorly water-soluble compounds and on the success of the formulation strategy mentioned before can be expected (Sugawara et al., 2005). The same hurdle is encountered in 10–20% of elderly people as they exhibit either diminished (hypochlorhydria) or no gastric acid secretion (achlorhydria), leading to basal gastric pH values >5.0 (Charman et al., 1997). In addition, relying merely on the gastro-intestinal acid–base sequence for enhancing bioactivity also holds the risk of uncontrolled precipitation of the drug compound at the site of absorption. Recently, the direct influence of gastric pH on atazanavir absorption was investigated. When lansoprazole, a proton pump inhibitor, was co-administered with atazanavir, a drastic reduction in bioavailability of atazanavir was observed (Tomilo et al., 2006). A similar behaviour was also reported for the bead-based capsules of Sporanox® which exhibit a significantly lower oral bioavailability of itraconazole when dosed to human subjects suffering from a reduced acidity of the stomach (Cartledge et al., 1997). This indicates that the co-dissolving HPMC phase cannot enhance the extent of absorption when hypochlorhydria is involved. It is therefore often recommended to co-administer an acidic soda beverage in patients who use the capsule formulation of itraconazole (Jaruratanasirikul and Kleepkaew, 1997).

The fact that basic drugs suffer from a decreased oral bioavailability when gastric acidity is reduced, urged formulation scientists to explore new strategies. In the case of itraconazole, absorption enhancement after oral administration was achieved when using a soluble formulation of itraconazole in hydroxypropyl-β-cyclodextrin solution; this may be particularly useful in HIV-infected patients. Pharmacokinetic data have shown that itraconazole absorption is enhanced by approximately 30% in healthy volunteers after administration of the cyclodextrin-based oral solution; thus, concurrent food and acidifying agents are no longer necessary as are recommended with the capsule formulation (Graybill et al., 1998). However, for a variety of reasons, including cost, formulation bulk and toxicology, the amounts of cyclodextrin that can be included in drug formulations is limited. This is further complicated by the fact that the complexation efficacy of cyclodextrins is, in general, very low and their molecular weight is rather high (Loftsson and Masson, 2004). Therefore other concepts to enhance the oral bioavailability of poorly water-soluble basic drugs which are independent of intraluminal pH sequence need to be explored.

Recently, we showed the ability of enhancing the release of itraconazole in simulated gastric fluid when loaded into ordered mesoporous silica (OMS) (Mellaerts et al., 2007). OMS exhibits a two-dimensionally ordered array of cylindrical pores of uniform size between 2 nm and 50 nm disposed parallel to each other and are separated by thin walls (Zhao et al., 1998). Such materials take profit from the wide variety of chemical compositions, porosities and morphologies that can be achieved via sol–gel chemistry using shape selective polymers and surfactants. Tailoring the fine structure of these inorganic frameworks enables researchers to construct devices which are adapted to fulfill the needs of molecular guest species, *in casu* therapeutic molecules (Ambrogio et al., 2007; Heikkila et al., 2007a,b). Originally, the focus of inorganic matrices has been on the development of slow release formulations (Vallet-Regi et al., 2007; Aerts et al., 2007), but the application as carrier for poorly water-soluble compounds is gaining momentum (Salonen et al., 2005; Kaukonen et al., 2007). The promising *in vitro* results obtained with OMS to enhance the dissolution of itraconazole have recently been confirmed by our group in comparative bioavailability studies in rabbits and dogs. Systemic exposure of itraconazole obtained with OMS as a carrier compared well to the marketed product Sporanox® in both species (Mellaerts et al., 2008).

The present study aims to explore whether OMS could be used as a carrier to create dosage form induced supersaturation of the weakly basic model compound itraconazole ($pK_a = 3.7$) in a neutral biorelevant medium. The ultimate aim was to explore whether OMS could be used to obtain a pH-independent dissolution enhancement with a concomitant increase in intestinal

absorption. This approach would increase reproducibility under highly varying conditions in the gastro-intestinal tract.

2. Materials and methods

2.1. Media for dissolution and transport experiments

Fasted state simulated intestinal fluid (FaSSIF) was prepared based on blank FaSSIF which is a phosphate buffer obtained by dissolving 0.696 g NaOH (BDH Laboratory Supplies, Poole, England), 7.908 g $NaH_2PO_4 \cdot H_2O$ (Merck, Darmstadt, Germany) and 12.372 g NaCl in 2 l of purified water (18.2 MΩ, Elga, TX, USA). The pH was adjusted to exactly 6.5 with 1 M NaOH. FaSSIF was created by adding 1.61 g of sodium taurocholate (ICN Biomedicals, Eschwege, Germany) and 5.90 ml of a solution of lecithin (YDS Chemicals, Heusden, Belgium) in chloroform (100 mg ml^{-1}) to approximately 200-ml blank FaSSIF. This mixture was heated to 80 °C for 15 min to remove all chloroform, after which a clear solution was obtained. Subsequently, blank FaSSIF was added up to a volume of 1 l. Some experiments were performed with MES-buffered FaSSIF to allow the addition of SGF (simulated gastric fluid, 0.1 M HCl containing 0.2 wt.% NaCl) without a significant pH change. MES [2-(*N*-morpholino)ethanesulfonic acid, Sigma–Aldrich, Steinheim, Germany] (977.34 mg), sodium taurocholate (179.1 mg) and lecithin solution (655.6 μl) were added to prepare 100 ml of MES-FaSSIF according to the same protocol as mentioned before. As receiver medium for the Caco-2 transport experiments, 0.2 wt.% TPGS (D-α-tocopherol polyethylene glycol 1000 succinate, Eastman, Anglesey, England) in transport medium (TM) (Hanks' balanced salt solution (HBSS) supplemented with glucose (to obtain a final concentration of 25 mM, Sigma–Aldrich, Steinheim, Germany) and Hepes-buffer (10 mM, pH 7.4, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, Lonza, Verviers, Belgium)) was used. To maintain sink conditions, TPGS was included in the medium added to the basolateral side of the Caco-2 cell monolayer (Deferme et al., 2002).

2.2. Synthesis of itraconazole loaded ordered mesoporous silica

Ordered mesoporous silica was synthesized according to the procedure described before (Mellaerts et al., 2007). Briefly, 6 g of triblock copolymer Pluronic P123 (BTC-Benelux, La Hulpe, Belgium) was dissolved in 180 g of 2 M HCl. This mixture was placed in an oil bath at 35 °C under magnetic stirring. An amount of 15.3 g of sodium silicate solution (>27 wt.% SiO_2, Riedel-de Haën, Seelze, Germany) was diluted with 45 g demineralised water. This mixture was added dropwise to the Pluronic P123 solution under vigorous stirring. Stirring was allowed to continue for another 5 min before switching to static synthesis conditions at 35 °C. After 24 h, the silica suspension was transferred into a teflon lined autoclave (K.U. Leuven Workshop) and placed in an oven for hydrothermal treatment at a temperature of 90 °C for another 48 h. Finally, the powder was washed on a 0.45-μm filter (Whatman Schleicher and Schuell, Dassel, Germany) with demineralised water, dried and calcined at 550 °C for 8 h under an ambient atmosphere to remove the triblock copolymer from the pores. Loading of OMS was performed by suspending OMS into an itraconazole (Janssen Pharmaceutica, Beerse Belgium) solution in methylene chloride (5 mg ml^{-1}). The mixture was agitated for 24 h using a rotary mixer (20 rpm, Snijders, Tilburg, The Netherlands). The initial OMS and itraconazole weight ratio amounted to 75 wt.% and 25 wt.%, respectively. Subsequently, the solvent was removed by evaporation and the powder was dried overnight at 35 °C. OMS loaded with itraconazole was heated to 100 °C for 5 min and placed under reduced pressure (10^{-3} bar) at 40 °C for another 48 h. Itraconazole loading was determined using a long-term release experiment during 7 days under sink conditions (0.1 M HCl, 0.5 wt.% sodium laurylsulfate (SLS; Certa s.a., Braine-l'Alleud, Belgium), $n = 5$).

2.3. Solubility and in vitro dissolution

2.3.1. Solubility determination

The solubility of itraconazole in FaSSIF was determined by weighing approximately 2 mg into an Eppendorf tube (1.5 ml) and adding 1 ml of freshly prepared FaSSIF. The tubes were shaken at 37 °C (Incubator-Shaker Series 25D, New Brunswick Scientific Co., United States of America) with a speed of 130 rpm to ensure the formation of a homogeneous suspension. After specific time intervals, the solid material was removed from the medium by centrifugation at 37 °C and 14,000 rpm for 10 min (Eppendorf 5804 R, Germany) ($n = 6$ per time point). The supernatant (400 μl) was diluted with mobile phase (1:1) and kept at 4 °C prior to analysis.

2.3.2. Solvent-induced supersaturation

Solvent-induced supersaturation was obtained by spiking FaSSIF with a concentrated solution of itraconazole (5 mM) in dimethylsulfoxide (DMSO; Acros Organics, Geel, Belgium). In this way, concentrations intended to range from 20 μM up to 75 μM were prepared. Due to precipitation, the real concentration cannot be predicted in advance. We therefore prefer to denote the considered samples throughout this manuscript with their theoretical concentrations intended initially. The final DMSO fraction in FaSSIF never exceeded 2 vol.%. Itraconazole supersaturation in FaSSIF was also generated based on a pH shift starting from a 100-μM itraconazole solution in SGF. After 1 h, 9 ml MES-FaSSIF was added to 1 ml of the above-mentioned solution. For both methods, supersaturation was characterized by determining the itraconazole concentration at specific time intervals. Samples were centrifuged at 14,000 rpm for 15 min to remove precipitated itraconazole. Supernatants were collected and immediately diluted with mobile phase (1:1) to prevent precipitation during storage and analysis.

2.3.3. Formulation-induced supersaturation

In order to study the formulation-induced supersaturation of itraconazole in biorelevant conditions, OMS loaded with itraconazole was suspended in 8 ml FaSSIF. The dissolution study was performed in test tubes of 12 ml under gentle agitation using a rotary mixer (Snijders, Tilburg, The Netherlands). The

amount of material in the dissolution medium was adjusted to eventually obtain a theoretical concentration of drug substance (75 μM). In the same way as with the solvent-induced supersaturation, the effect of prior acidic dissolution was evaluated by suspending the dosage form into 1 ml SGF. After 1 h, the medium was converted into FaSSIF with the addition of 9 ml MES-FaSSIF. At specific time intervals, samples were collected and the medium was filtered through a 0.45-μm PTFE membrane. Prior to analysis by HPLC, samples were diluted with methanol (1:1) to prevent precipitation during storage and analysis.

2.3.4. HPLC assay

Samples of the *in vitro* dissolution study were assayed using an isocratic HPLC method. The HPLC system consisted of a LaChrom® L-7100 HPLC pump, an autosampler model L-7200 equipped with a 100-μl loop, a UV detector model L-7420 set at 260 nm, and an Interface D-7000 (all Merck, Darmstadt, Germany). UV signals were monitored and peaks were integrated using the D-7000 HSM software. The separation of itraconazole was performed on an RP-18 150 mm × 4.6 mm 5 μm Hypersil silica column (Thermo Electron Corporation, Waltham, United States of America) at room temperature. The mobile phase consisted of acetonitrile:tetrabutyl ammonium hydrogen sulfate 0.01N (55:45, v/v), and was filtered through a 0.45-μm PTFE membrane and degassed by ultrasonication before use. The flow rate amounted to 1.5 ml min^{-1}. The standard curves were linear over the concentration range of 0.0001–3 mg ml^{-1}.

2.4. Differential scanning calorimetry

In order to study the nature of the precipitate formed during solubility determinations, DSC analysis was performed. The collected precipitate was washed with demineralised water and dried for 24 h at room temperature in the presence of silica gel. DSC analysis was carried out on a DSC 2920 (TA Instruments, Leatherhead, United Kingdom). The sample was heated from 20 °C to 200 °C at 2 °C min^{-1}. Indium was used to calibrate the temperature scale and the enthalpic response. The samples were analyzed in open aluminum sample pans (TA Instruments, Leatherhead, United Kingdom).

2.5. Caco-2 cell monolayer transport study

2.5.1. Cell culture

Caco-2 cells were purchased from Cambrex Biosciences (Walkersville, MD). Caco-2 cells were grown in 75 cm^2 culture flasks at 37 °C in an atmosphere of 5% CO_2 and 90% relative humidity. Cells were passaged every 3–4 days (at 70–80% confluence) at a split ratio of 1:7. Cells were negative for Mycoplasma infection.

2.5.2. Transport experiments

For transport experiments, Caco-2 cells were plated at a density of 88,500 cells/insert on Costar® Transwell membrane inserts (3-μm pore diameter, 12-mm diameter; Corning Inc., NY). Confluence was reached within 3–4 days after seeding and the monolayers were used for the experiments 18–19 days post-seeding. Cell passages between 50 and 85 were used in the experiments. Transepithelial electrical-resistance values (TEER values) were measured with an EndOhm Voltohmmeter (WPI, Aston, England). Only monolayers with initial TEER values higher than 200 Ω cm^2 were used. All volumes amounted to 0.5 ml at the apical side of the monolayer and 1.5 ml at the basolateral side. After rinsing the monolayers three times with TM, a pre-incubation step (30 min) with TM (control) was performed.

After measuring TEER values, transport was initiated by adding to the donor compartment: (a) an itraconazole solution in FaSSIF (20 μM, 40 μM and 75 μM) obtained by DMSO spiking, (b) a suspension of OMS loaded with itraconazole in a selected medium or (c) Sporanox® pellets in FaSSIF. The amount of itraconazole added by a formulation was kept constant at a theoretical value to eventually generate a donor solution of 75 μM. Samples (100 μl) were taken from the acceptor compartment after 30 min, 60 min and 90 min and replaced with 100 μl fresh receiver medium. During the experiments, 0.2% TPGS was included in the TM added to the basolateral compartment to install sink conditions. To verify whether this medium can be considered as a biorelevant basolateral medium, transport was also studied using rabbit plasma as the receiver medium. When a 75-μM supersaturated solution was applied under these conditions, total transport after 90 min was comparable to the one obtained when 0.2% TPGS in TM is used (data not shown), suggesting that this medium can be considered as a valid biorelevant substitute. The samples were diluted with methanol (1:1) and itraconazole concentration was determined with the HPLC method mentioned above. TEER values were measured again at the end of the experiment and were higher than 95% of the initial value. As an additional control of the monolayer integrity, sodium fluorescein flux was measured at the end of the experiment. Briefly, sodium fluorescein (1 mg ml^{-1}) was added to the apical compartment and after 60 min, samples were taken from the basolateral compartment, followed by TEER measurement. The amount of sodium fluorescein appearing in the basolateral compartment was measured by UV spectrophotometry (Uvikon 810P spectrophotometer, Kontron Instruments, Watford, England) at 490 nm. Sodium fluorescein flux values across the monolayers were below 0.6% h^{-1} cm^{-2}. None of the test conditions affected the integrity of the tight junctions during the time period studied (based on TEER measurements and sodium fluorescein flux).

2.6. In situ perfusion transport study

2.6.1. Experimental set-up

The rats used in this study are purpose-bred. These rats are housed – according to the Belgian and European laws, guidelines and policies for animal experiments, housing and care – in the Central Animal Facilities of the University. These facilities have the obligatory accreditation of the authorised Belgian Ministry and are registered under license number LA1210261. Approval for this project was granted by the Institutional Ethical Committee for Animal Experimentation. *In situ* perfusion experiments were performed based on a previously described method

(Annaert et al., 2000). Male Wistar rats of approximately 350 g (Janvier, France) were anaesthetized with a ketamin (Anesketin, Eurovet, Heusden, Belgium)–xylazin (Xyl-M 2%, VMD, Arendonk, Belgium) mixture (87.5 mg kg^{-1} and 8.75 mg kg^{-1}, respectively). The left jugular vein was cannulated with a heparinized (50 IU ml^{-1}) polyethylene cannula (1.02-mm outer diameter; Portex, Kent, UK) for blood supply from a donor rat during the perfusion experiment. A laparotomy was performed and the small intestine was exposed. A segment of the ileum (4–10 cm) was isolated by inserting two glass cannulas (4-mm o.d., 3-mm i.d.) at the proximal and distal end of the segment. Polyethylene tubing (6.5-mm o.d., 3.1-mm i.d.) was connected to the inlet cannula. The intestinal content was removed by perfusing the segment with pre-warmed TM (38 °C) at a flow rate of 3 ml min^{-1}. The perfusion pump (Minipuls3, Gilson, Middleton, USA) was placed between the reservoir and the inlet cannula. After pre-incubation of the intestine with TM, the mesenteric vein draining the isolated part of the ileum was cannulated using the top end (1 cm) of a catheter (Insyte-W® 0.7 mm × 19 mm, Beckton Dickinson, Salt Lake City, UH). The cannula was secured with a knot and connected to a piece of 40 cm silastic tubing (0.64-mm internal diameter; 1.19-mm outer diameter, Helix Medical, USA).

2.6.2. *In situ perfusion experiments with itraconazole*

The flow rate of the perfusate amounted to 1 ml min^{-1}. The perfusion experiments were carried out using FaSSIF with (a) a saturated itraconazole solution, (b) a supersaturated itraconazole solution generated by solvent spiking with DMSO, (c) a suspension of OMS loaded with itraconazole and (d) Sporanox® pellets. The maximal theoretical concentration of conditions b–d amounted to 75 μM. At the beginning of the perfusion with the itraconazole solution, blood was collected from the mesenteric vein and donor blood supply (supplemented with ketamin–xylazin (12.5 mg kg^{-1} h^{-1} and 1.25 mg kg^{-1} h^{-1}, respectively)) was initiated via the jugular vein at a rate of 0.3 ml min^{-1} using a syringe pump (Pilot A2, Fresenius Vial, Grenoble, France). Experiments with media a and b as the perfusate were performed under an open-loop set-up. Experiments with the formulations (c and d) were performed under a closed-loop (perfusate was recirculated) system. Blood from the mesenteric vein was collected in heparinized tubes over 5-min time intervals for 60 min. In addition, samples were taken from the perfusion medium at 0–20–40–60 min after the start of the perfusion. The collected blood samples were centrifuged at 4000 rpm for 10 min at 4 °C to separate the cells from the plasma within 30 min after collection. Plasma samples were frozen and stored prior to analysis at −20 °C. The collected perfusate samples were centrifuged for 15 min at 37 °C. 400 μl supernatant were mixed with 400 μl mobile phase (78% methanol, 22% buffer 25 mM sodium acetate pH 3.3) and stored at 4 °C prior to analysis.

2.6.3. *HPLC assay*

Plasma concentrations of itraconazole were determined by a validated HPLC method. To 1 ml of plasma, 100 μl of the internal standard solution was added (R051012 (Janssen Pharmaceutica), 2.5 μM in 0.2 M HCl). After addition of 500 μl 2 M NaOH, itraconazole and hydroxyitraconazole were extracted with 4 ml diethyl ether. Following centrifugation at 4000 rpm for 5 min, the upper organic layer was transferred into a fresh tube. The organic solvent was evaporated under a gentle stream of air and the extraction residue was dissolved in 200 μl methanol:water mixture (50:50, v/v), of which 99.5 μl was injected into the HPLC system. Concentrations of itraconazole and hydroxyitraconazole were determined using an isocratic HPLC method. The HPLC system (Merck-Hitachi, Darmstadt, Germany) used for the analysis of the plasma samples consisted of an Elite LaChrom L-2130 HPLC pump, an autosampler model L-2200 and a UV detector model L-2400. Separations were achieved using a Novapak C-18, 4 μm, under radial compression. The mobile phase consisted of methanol:25 mM sodium acetate pH 3.3 (78:22, v/v). The mobile phase was filtered through a 0.45-μm PTFE membrane before use. The flow rate of the mobile phase with online degassing was maintained at 1.75 ml min^{-1} and the effluent was monitored at a wavelength of 265 nm. Itraconazole, hydroxyitraconazole and R051012 were eluted with retention times of 4.5 min, 8.0 min and 11.5 min, respectively. The standard curves were linear over the concentration range of 7.8–500 nM. The intraday reproducibility values, expressed as the R.S.D., were less than 7.3% for itraconazole and less than 5.4% for hydroxyitraconazole over the concentration range studied ($n = 6$).

3. Results and discussion

3.1. *Solvent-induced supersaturation*

Intraluminal supersaturation can be expected when a poorly water-soluble basic compound is first dissolved in the acidic environment of the stomach and subsequently released into the small intestine. This transition was simulated starting from a SGF solution of itraconazole (100 μM): after an initial residence of 1 h at pH 1.2, the medium was converted into FaSSIF and itraconazole concentrations were determined to study the ability to create intraluminal supersaturation. Because this pH shift was accompanied with a 10-fold dilution, the maximum theoretical concentration in FaSSIF amounted to 10 μM. The resulting concentration–time profile is depicted in Fig. 1. After the pH shift to 6.5, the itraconazole concentration immediately dropped to 7.85 ± 0.02 μM; after 30 min, the concentration amounted to 2.82 ± 0.02 μM. This decrease can be attributed to the partial precipitation of itraconazole. Following the initial drop, the concentration increased again to achieve approximately 4 μM for the next 3 h. Despite precipitation, itraconazole concentrations readily exceed the thermodynamic solubility of itraconazole in FaSSIF. The equilibrium solubility value was determined during an equilibration period for up to 1 week and amounted to 0.40 ± 0.03 μM (presented as a dotted horizontal line in Fig. 1). The extent of supersaturation can be expressed as the actual concentration of itraconazole over its equilibrium solubility (*c*/*s*). Here, the minimal extent of supersaturation amounted to 7.1 and was observed 30 min after the pH shift. The fact that, in FaSSIF (pH 6.5), a supersaturated state of itraconazole was

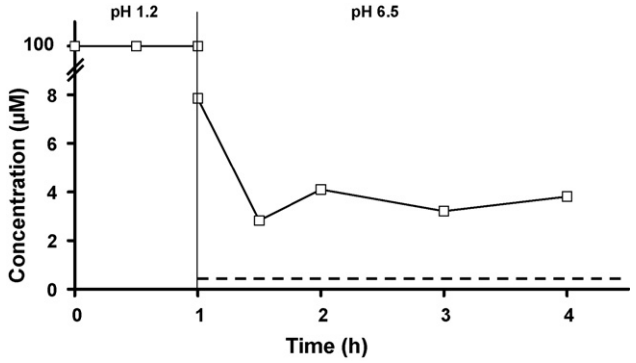

Fig. 1. Concentration–time profile of itraconazole in SGF (pH 1.2) for 1 h (100 μM) and subsequently in FaSSIF (pH 6.5) for 3 h (10 μM). Average ± S.D., S.D. smaller than symbols, $n = 4$. The equilibrium solubility of crystalline itraconazole in FaSSIF is presented as a dotted line (- - -).

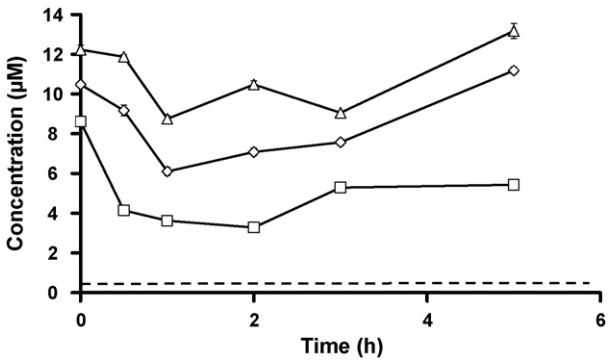

Fig. 2. Concentration–time profile of itraconazole in FaSSIF obtained by the solvent shift method starting from a stock solution of itraconazole in DMSO (5 mM). Theoretical concentrations amounted to 20 μM (□), 40 μM (◊) and 75 μM (△). Average ± S.D., S.D. smaller than symbols, $n = 4$. The equilibrium solubility of crystalline itraconazole in FaSSIF is presented as a dotted line (- - -).

maintained during at least 3 h is indicative for the presence of a mechanism which inhibits nucleation and crystal growth. The intestinal medium FaSSIF indeed contains two surface active compounds, lecithin and sodium taurocholate. Surfactants in general can solubilise materials through micellar encapsulation but can also alter the surface tension at the crystal–medium interface (Constantidines et al., 2006; Rangel-Yagui et al., 2005), providing a possible mechanism to explain the maintenance of the supersaturated state of itraconazole in FaSSIF. This also suggests that there is a reduced need to include solubility enhancing and stabilizing agents into formulations which aim to create supersaturation of basic compounds in intraluminal media as natural surface active components are present.

The formation of a supersaturated solution in FaSSIF when using the pH shift method is a time-consuming laboratory practice. Therefore, we explored the possibility to create supersaturated itraconazole solutions by means of a solvent shift method using DMSO as the primary solvent. This would enable us to create and investigate the extent of supersaturation directly in FaSSIF without the requirement of an acidic dissolution step. Three different theoretical concentrations in FaSSIF (20 μM, 40 μM and 75 μM) were prepared starting from a concentrated itraconazole solution in DMSO (5 mM). The concentration of dissolved itraconazole was monitored for 5 h and the resulting concentration–time profiles are presented in Fig. 2. When the actual itraconazole concentration is measured directly after solvent shifting, the obtained values are much lower than the theoretical concentrations: 8.62 ± 0.06 μM, 10.48 ± 0.06 μM and 12.23 ± 0.23 μM compared to 20 μM, 40 μM and 75 μM, respectively. This decrease was also observed using the pH shift method and can be attributed to an immediate partial precipitation of itraconazole. After centrifugation, the nature of this precipitate was investigated by DSC analysis: the precipitate consisted of an amorphous (66 wt.%), as well as a crystalline itraconazole solid phase (34 wt.%). After this initial precipitation, itraconazole concentrations readily exceed the thermodynamic solubility concentration of itraconazole in FaSSIF for at least 5 h. During this period, the extent of supersaturation (*c*/*s*) was at least 8.2, 15.3 and 21.9 when starting from a solution with a theoretical concentration of 20 μM, 40 μM and 75 μM, respectively. This comparison evidences that a supersaturated itraconazole solution in FaSSIF can be generated by spiking the neutral aqueous medium FaSSIF with a concentrated itraconazole in DMSO solution and that the extent of supersaturation depends on the initial amount of itraconazole used. Preliminary experiments excluded the influence of the DMSO content on itraconazole solubility (data not shown). The extent of supersaturation does not increase linearly with the amount of itraconazole spiked in FaSSIF, suggesting that the maximum attainable supersaturation will be limited. The slight concentration increase observed following the initial precipitation could presumably be attributed to a partial dissolution of the formed precipitate. The data presented so far evidence that supersaturation can be created by a pH shift approach, as well as by a solvent shift approach. In a next step, we wanted to explore whether supersaturation in FaSSIF could also be created by a formulation approach.

3.2. Formulation-induced supersaturation

Recently, we showed the ability to enhance the release of itraconazole in SGF using ordered mesoporous silica (Mellaerts et al., 2007). The release experiments with OMS as carrier were performed under acidic conditions as this environment was thought to be favourable in order to obtain a fast liberation of the itraconazole molecules from OMS. The sharp pH shift upon arrival in the small intestine may, however, result in precipitation of itraconazole that was dissolved in the stomach, thus jeopardizing the absorption process and decreasing oral bioavailability. In the present study, the release of itraconazole from OMS was assessed under pH shift conditions in order to better reflect the *in vivo* situation. One hour after the addition of itraconazole loaded OMS to SGF (100 μM), the medium was converted to FaSSIF. Release profiles are presented in Fig. 3. In parallel to the release of itraconazole from OMS, the performance of the marketed product Sporanox® was also examined under the above-mentioned conditions at a dose which would eventually also result in a theoretical concentration of 100 μM. After a 1-h residence time in SGF, 34.66 ± 2.62% of the amount of itraconazole loaded into OMS was released. Comparison of

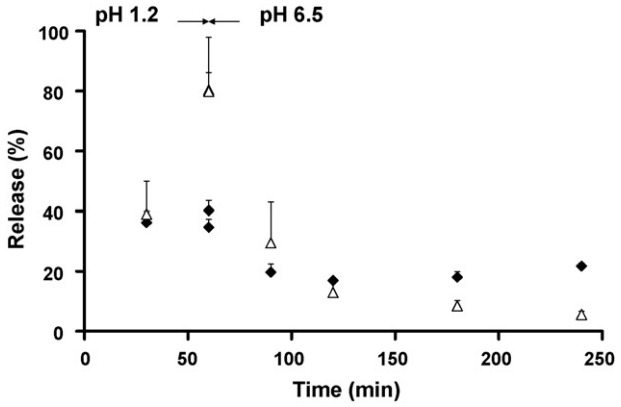

Fig. 3. Release profile of itraconazole from OMS (♦) and Sporanox® (△) in SGF (pH 1.2) for 1 h (100 μM) and subsequently in FaSSIF (pH 6.5) for 3 h (10 μM). Average ± S.D., $n = 3$.

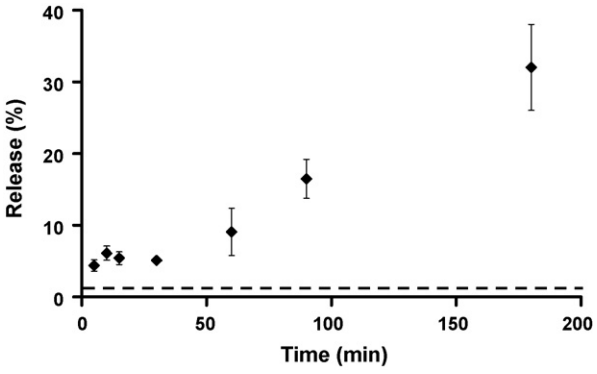

Fig. 4. Release profile of itraconazole liberated from OMS in FaSSIF (pH 6.5). The maximal attainable concentration amounted to 75 μM. Average ± S.D., $n = 3$. The equilibrium solubility of crystalline itraconazole in FaSSIF is presented as a dotted line (- - -).

OMS with Sporanox® for the first hour in SGF, clearly shows the solubility enhancing properties of the water-soluble HPMC present in the marketed product: 80.42 ± 17.50% of the itraconazole present was liberated into the release medium. The absolute concentration of itraconazole dropped by a factor of 10 due to the dilution upon conversion of the medium to FaSSIF. After 30 min, the amount of dissolved itraconazole when using Sporanox®, decreased from 80.42 ± 17.50% to 29.46 ± 13.54%, and even further to 5.58 ± 1.05% after 3 h, clearly demonstrating that precipitation occurred after the pH shift; the lowest concentration of itraconazole still represented a supersaturated state ($c/s = 10.5$). When the acidic medium containing itraconazole loaded OMS was converted into FaSSIF, precipitation was less severe, as reflected in the fact that, 30 min after the pH shift, the amount of dissolved itraconazole decreased from 34.66 ± 2.62% to 19.71 ± 2.69%, and even slightly increased up to 21.70 ± 1.01% after 3 h. The extent of supersaturation (c/s) obtained when OMS was used as a carrier therefore varied between 31.7 and 39.5 in FaSSIF. These observations were unexpected, because several authors did point out the necessity to include a stabilizing polymer which can extend the time the compound remains in the supersaturated state; such polymers (e.g. HPMC) are supposed to prevent recrystallization or particle growth in solution through hydrogen-bonding (Raghavan et al., 2003), an increased medium viscosity or by adsorbing on the surface of solid particles blocking the addition of other solute onto the surface (Raghavan et al., 2001). OMS does not exhibit such a solubility enhancing effect; therefore, itraconazole released from OMS was expected to be more prone to precipitation when compared to the HPMC containing Sporanox® pellets. Intriguingly, the opposite is observed: when OMS is used, the itraconazole concentration even increases towards the end of the experiment. Such increase could not be observed in the case of Sporanox®.

Both solid formulations, OMS and Sporanox®, were able to maintain a certain extent of supersaturation after the pH shift. However, several diseases (e.g. AIDS) are accompanied with a reduced acidity of the stomach which is expected to impede a formulation strategy for poorly water-soluble basic drugs which requires a sequential acidic–neutral environment for optimal performance. We therefore explored the ability of both formulations to release itraconazole in the absence of a pH gradient. Upon addition of itraconazole from Sporanox® directly into FaSSIF, the amount of itraconazole released was only 1.21 ± 0.02% after 120 min which clearly illustrates the reduced performance of the marketed solid dispersion in the absence of a preceding acidic environment. This is in agreement with several studies from other authors that observed a reduction of the performance when the acidity in the stomach was reduced. A completely different profile was obtained when itraconazole loaded OMS was added directly into FaSSIF (Fig. 4). After an initial phase during which approximately 5% of itraconazole is released from its carrier, the amount of itraconazole released rises quickly reaching values of 16.5 ± 2.7% and 32.0 ± 2.7% after 90 min and 120 min, respectively. The mechanism underlying this peculiar release curve remains yet unknown. Although, the amount of itraconazole initially loaded into OMS would eventually result in a concentration of 75 μM, 5% corresponds to a concentration of 3.8 μM, which is much higher than the thermodynamic equilibrium concentration (depicted in Fig. 4 as a dotted horizontal line). From this comparison it can be concluded that OMS is able to create a pH-independent supersaturated state of itraconazole in FaSSIF. This finding clearly indicates that OMS has the potential to release loaded drug in conditions where the gastric acidity of the stomach is reduced. In this way, the pH dependence of drug solubility and formulation performance can be circumvented.

3.3. Caco-2 transport experiments

In a next set of experiments, we wanted to explore whether supersaturation in a biorelevant medium was accompanied with increased absorption. Based on the drug's thermodynamic activity, an enhanced flux at the absorption site can indeed be expected when supersaturated solutions are created at the donor side. This possible beneficial effect of supersaturation on intestinal absorption was first explored by using the solvent shift method. Three donor concentrations of itraconazole in FaSSIF were prepared: a saturated itraconazole solution, a 10 μM supersaturated condition and a 75 μM supersaturated

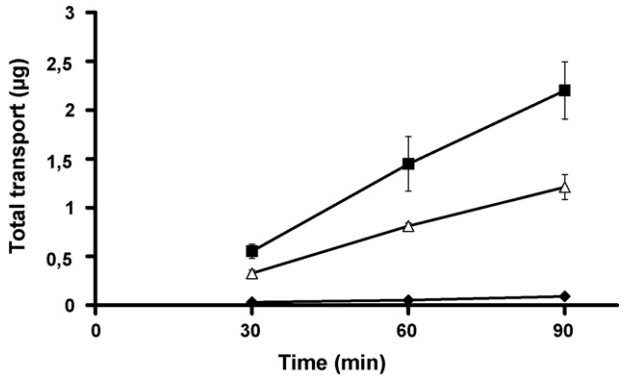

Fig. 5. Total transport (μg) of itraconazole in function of time across a Caco-2 cell monolayer starting from a saturated itraconazole solution (♦), a 10-μM supersaturated solution (△) and a 75-μM supersaturated solution in FaSSIF (■). Average ± S.D., $n = 3$.

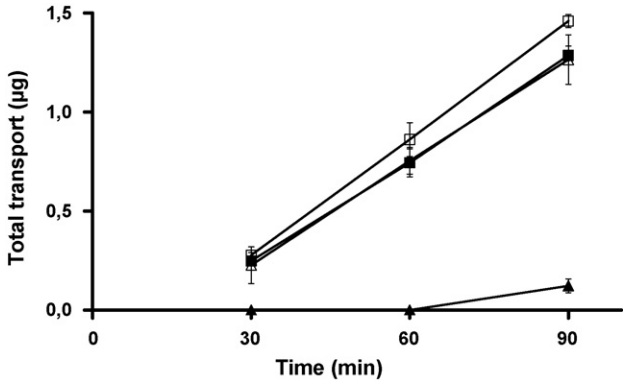

Fig. 6. Total transport (μg) of itraconazole in function of time across a Caco-2 cell monolayer. Donor media: an OMS suspension with a theoretical itraconazole concentration of 75 μM in FaSSIF without (■) and with (□) a prior acidic 1 h dissolution step, a suspension of Sporanox® pellets with a theoretical itraconazole concentration of 75 μM in FaSSIF without (▲) and with (△) a prior acidic 1 h dissolution step. Average ± S.D., $n = 3$.

condition. Transepithelial transport is presented in Fig. 5. The concentration of itraconazole in the donor compartment is not constant due to precipitation which occurs after solvent spiking in the intestinal medium (Fig. 2); consequently no permeability values could be calculated and transport was expressed as the total amount of itraconazole appearing at the basolateral side of the Caco-2 cell monolayer. The saturated solution generated a total transport of 0.09 ± 0.00 μg after 90 min. When the 10 μM and 75 μM supersaturated itraconazole condition was used in the donor compartment, total itraconazole transport increased drastically up to 1.21 ± 0.13 μg and 2.20 ± 0.29 μg, respectively. In Fig. 2, it is illustrated that the extent of supersaturation created for the 75 μM conditions is approximately 25. This illustrates that the extent of supersaturation is translated into a similar increase in transepithelial transport.

Subsequently, we investigated whether it is possible to achieve a similar transport enhancement for itraconazole based on the biocompatible formulation strategy with OMS as a carrier. The results from transport experiments across a Caco-2 cell monolayer were also compared with the marketed product Sporanox®. As the Caco-2 monolayer is not compatible with acidic medium, the acidic–neutral pH shift which is normally encountered in healthy people, was simulated by an acidic dissolution step, prior to conversion into FaSSIF; this medium was subsequently added to the donor compartment of the Caco-2 system. The amount of itraconazole present in the loaded OMS or Sporanox® used would eventually result in a theoretical maximal concentration of 75 μM. Transport was monitored in function of time and is presented in Fig. 6. Total transport of itraconazole was slightly higher for OMS compared to Sporanox® amounting to 1.46 ± 0.03 μg and 1.26 ± 0.12 μg after 90 min, respectively. This is in agreement with the *in vitro* dissolution profiles presented in Fig. 3 which evidenced a higher extent of supersaturation after pH was shifted in the case of OMS.

As mentioned before, a reduced gastric acidity may jeopardize the pharmaceutical performance of a solid dosage form if the formulation requires an acidic–neutral pH shift. The development of a solid dosage form that maintains its performance independent of changes in gastric pH is a great challenge to formulation scientists. Based on the promising *in vitro* results when using OMS (Fig. 4), evidencing the generation of a supersaturated state in FaSSIF, we examined whether it is possible to enhance transport of itraconazole independent from prior dissolution in acidic medium. Two different amounts of itraconazole loaded OMS eventually resulting in a theoretical concentration of 10 μM and 75 μM in FaSSIF were chosen as the donor conditions and total transport was monitored in function of time. Results from this experiment are presented in Fig. 7 and comparison was made with a saturated solution of itraconazole in FaSSIF. After 90 min, the formulation-induced supersaturation resulted in a total transport of itraconazole of 0.42 ± 0.03 μg and 1.31 ± 0.10 μg for the 10 μM and 75 μM donor conditions, respectively. As compared to the total transport achieved when using a saturated itraconazole solution (0.09 ± 0.00 μg), it can be concluded that OMS as a carrier results in a remarkable increased transport of itraconazole across the Caco-2 cell monolayer. Transport values obtained by the formulation approach were lower than those obtained by solvent spiking, which may be attributed to the fact an additional release step is involved. In any case, OMS is able to create an *in vitro* supersaturated state and to

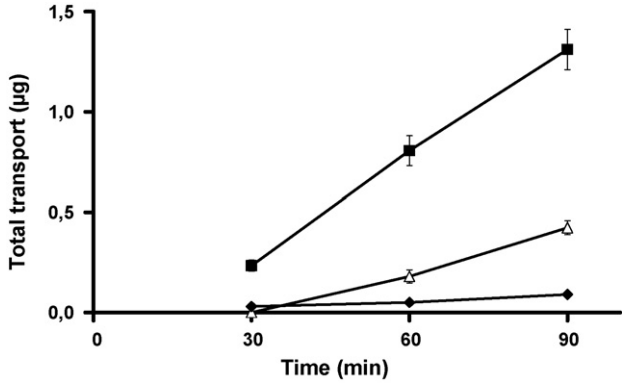

Fig. 7. Total transport (μg) of itraconazole in function of time across a Caco-2 cell monolayer starting from a saturated itraconazole solution (♦), an OMS suspension with a theoretical itraconazole concentration of 10 μM (△) and an OMS suspension with a theoretical itraconazole concentration of 75 μM in FaSSIF (■). Average ± S.D., $n = 3$.

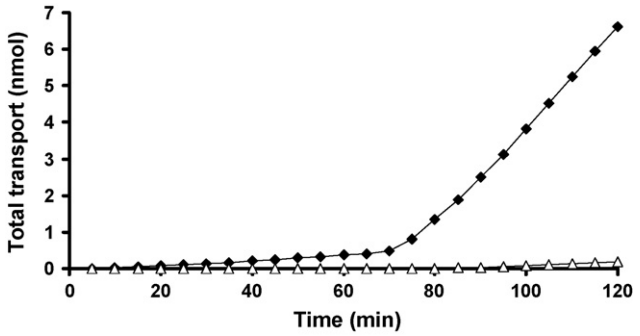

Fig. 8. Total transport in function of time for an *in situ* perfusion experiment using a saturated itraconazole in FaSSIF solution (first 60 min) and a supersaturated itraconazole solution (with a theoretical concentration of 75 μM). Itraconazole (♦) and OH-itraconazole (△) are presented.

enhance transport of itraconazole without a prior acidic dissolution step. The performance of the marketed product Sporanox® under similar conditions is illustrated in Fig. 6. Transport generated by using Sporanox® pellets amounted to 0.12 ± 0.03 μg after 90 min which is inferior as compared to 1.29 ± 0.05 μg for the OMS as a carrier. The mechanism of OMS to enhance the release of itraconazole has been suggested to be a competitive sorption of water to the surface of the porous structure (Mellaerts et al., 2007). In this view, OMS behaves as an inert matrix and releases itraconazole without changing any properties of the aqueous medium. In contrast, Sporanox® is based on a different approach involving release in the acidic environment of the stomach accompanied with an increase in viscosity of the medium due to the co-dissolving HPMC-phase. This allows the solubility of itraconazole and subsequently the extent of absorption to be enhanced after arrival in the small intestine. When no acidic dissolution is present prior to absorption, the biopharmaceutical performance of Sporanox® is drastically reduced. In contrast, OMS as a carrier maintains its ability to boost the transepithelial flux of the basic drug itraconazole.

3.4. *In situ* transport experiments

To further validate these findings and to confirm that OMS is a carrier that retains its pharmaceutical performance when a reduced gastric acidity is involved, we conducted transport experiments in a rat *in situ* perfusion set-up. This set-up better resembles the *in vivo* situation than the Caco-2 system.

Absorption was first explored using a saturated solution and a supersaturated solution obtained by the solvent shift approach. A typical cumulative concentration–time profile is presented in Fig. 8, illustrating that a drastic increase in absorption is obtained when switching the perfusion medium from a saturated to a supersaturated solution. This result is in line with the results obtained from the Caco-2 experiments and confirms that supersaturation is a key strategy to enhance the extent of absorption of poorly water-soluble compounds. The concentration of the main metabolite hydroxyitraconazole was also determined, but no significant concentrations could be detected, indicating that metabolism at the site of absorption is negligible.

Table 1
Total cumulative amount of itraconazole transported into the mesenteric vein upon perfusion of the small intestine with different perfusate media after 60 min

Perfusate medium	Total transport after 60 min (nmol cm^{-1})
Saturated solution	0.03 ± 0.01
Supersaturated solution[a]	0.50 ± 0.32
OMS suspension[a]	0.70 ± 0.09
Sporanox® suspension[a]	0.29 ± 0.01

[a] The theoretical concentration amounted to 75 μM in all three cases.

Subsequently, we evaluated the performance of OMS as a biocompatible matrix to enhance transport by generating intraluminal supersaturation. The performance of Sporanox® was also investigated and compared with OMS. No preceding dissolution in an acidic environment was allowed thus simulating the condition in which the gastric acidity is reduced. Total transport of itraconazole into the mesenteric blood is corrected for the length of the perfusated part of the intestine and values are presented in Table 1. With OMS, a total absorption of 0.70 ± 0.09 nmol cm^{-1} was achieved after 60 min which is comparable with the solvent-induced supersaturated solution (0.50 ± 0.32 nmol cm^{-1}). When a saturated solution is used as the perfusate, total transport amounted to only 0.03 ± 0.01 nmol cm^{-1}. This very clearly illustrates that OMS as a formulation is able to preserve its dissolution enhancing properties under conditions with a reduced gastric acidity. Under the same conditions, the marketed product Sporanox® has an inferior performance when compared to OMS. A total transport of only 0.29 ± 0.01 nmol cm^{-1} was recorded with Sporanox® which confirms the statement that the marketed solid dispersion suffers from a reduced performance when hypochlorhydria is involved.

4. Conclusions

In this study, we explored whether it is possible to generate and maintain a supersaturated state of itraconazole in FaSSIF and investigated subsequently its beneficial effects on transepithelial transport.

The creation of a supersaturated state of itraconazole in FaSSIF can be obtained when a concentrated organic or acidic itraconazole solution is converted into FaSSIF. Such a sudden solvent shift makes it possible to exceed the thermodynamic solubility. Starting from a supersaturated solution in FaSSIF, it was possible to boost transepithelial flux of itraconazole drastically in the Caco-2 system, as well as in the *in situ* perfusion model.

Ordered mesoporous silica was evaluated as a biocompatible formulation approach to provoke the same positive effect on intestinal transport. When a preceding acidic dissolution step was included to simulate the passage through the stomach, total transport across a Caco-2 cell monolayer was comparable for the OMS formulation, the solvent shift approach and the reference product Sporanox®. Because the majority of poorly water-soluble drugs are basic, this pH sequence is often a perquisite to allow for sufficient oral bioavailability. Skipping this pH sequence, resulted in a reduced performance of Sporanox®, in

the Caco-2 system as well as the *in situ* perfusion model. This is attributed to the fact that the mechanism of Sporanox® is designed in such a way that preceding acidic conditions are needed to create intraluminal supersaturation. In contrast, the *in vitro* profiles generated by OMS illustrate the excellent capability of exceeding the thermodynamic equilibrium concentration of itraconazole in FaSSIF in the absence of a pH shift. As a consequence, transport over a Caco-2 cell monolayer and in an *in situ* system was increased drastically to values comparable to the ones obtained by the solvent shift induced supersaturation. OMS appears to be a promising strategy for the delivery of especially basic low solubility compounds in patients suffering from hypochlorhydria; the pH-independency may also result in better reproducibility in systemic exposure.

Acknowledgements

The work was supported by a grant from the Research Foundation-Flanders, a KULeuven research grant (OT), a KULeuven interdisciplinary research project (IDO) and an industrial research fund (IOF). We also thank S. Clarysse and A. Bijnens for the assistance during the Caco-2 cell monolayer transport experiments.

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