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Supersaturation and Precipitation of Posaconazole Upon Entry in the Upper Small Intestine in Humans

Bart Hens [1], Joachim Brouwers [1], Maura Corsetti [2], Patrick Augustijns [1,*]

[1] Drug Delivery & Disposition, KU Leuven, Belgium
[2] Translational Research Center for Gastrointestinal Disorders (TARGID), KU Leuven, Belgium

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ABSTRACT

The purpose of this study was to explore gastrointestinal dissolution, supersaturation and precipitation of the weakly basic drug posaconazole in humans, and to assess the impact of formulation pH and type on these processes. In a cross-over study, two posaconazole suspensions (40 mg dispersed in 240 mL water at pH 1.6 and pH 7.1, respectively) were intragastrically administered; subsequently, gastric and duodenal fluids were aspirated. In parallel, blood samples were collected. Additionally, posaconazole was intragastrically administered as a solution (20 mg in 240 mL water, pH 1.6). When posaconazole was administered as an acidified suspension, supersaturated duodenal concentrations of posaconazole were observed for approximately 45 min. However, extensive intestinal precipitation was observed. Administration of the neutral suspension resulted in subsaturated concentrations with a mean duodenal $AUC_{0\text{-}120\ min}$ and C_{max} being approximately twofold lower than for the acidified suspension. The mean plasma $AUC_{0\text{-}8\ h}$ of posaconazole was also twofold higher following administration of the acidified suspension. Similar to the acidified suspension, significant intestinal precipitation (up to 92%) was observed following intragastric administration of the posaconazole solution. This study demonstrated for the first time the gastrointestinal behavior of a weakly basic drug administered in different conditions, and its impact on systemic exposure.

Introduction

Exploring gastrointestinal drug and formulation behavior and its eventual effect on drug absorption is extremely challenging in terms of various simultaneously ongoing processes, including dissolution, degradation, precipitation, and permeation. Understanding these processes is very important in order to improve the value of predictive *in vitro* biopharmaceutical tools, or to define relevant input parameters for physiologically based pharmacokinetic modeling. In the present study, we specifically focused on the characterization of intestinal precipitation of a poorly soluble weak base by comparing the intraluminal behavior of a drug solution versus 2 drug suspensions in humans.

Posaconazole was selected as basic model compound. For almost 10 years, posaconazole is registered as one of the most potent triazole antifungal agents. Its antifungal activity against *Zygomyces* spp. has been reported to be more pronounced *in vitro* compared with itraconazole.[1] Moreover, by inhibiting the fungal cytochrome P450-dependent enzyme 14α-demethylase, its antimycotic activity can be expanded to other fungi, including *Aspergillus* spp. as such, *Blastomyces* spp., *Coccidiomyces* spp., *Candida* spp., *Fusarium* spp., *Histoplasma* spp., and *Cryptococcus* spp.[2-7] With respect to *in vivo* disposition of posaconazole, several reports indicate extensive distribution in the body after oral intake of the marketed suspension Noxafil® (40 mg/mL), resulting in 40-fold higher tissue levels compared with serum levels.[8,9]

Complete recovery from invasive fungal infections depends on the extent of intestinal absorption of posaconazole. In fasting conditions, fluctuations in systemic exposure after oral administration have warranted therapeutic drug monitoring in immunocompetent patients.[10,11] Characterized by a low aqueous solubility but high intestinal permeability, posaconazole (molecular weight = 700.78 g/mol; cLog *P* 4.6) can be classified as a BCS 2 compound.[12,13]

This work is dedicated to the memory of my father, Patrick Hens (1957-2015), and to the memory of Dr. Marcus Brewster (1957-2014).

* *Correspondence to*: Patrick Augustijns (Telephone: +32-16-330301; Fax: +32-16-330305).
E-mail address: patrick.augustijns@pharm.kuleuven.be (P. Augustijns).

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Although no specific solubilization technology is being used for the suspension, relatively high gastric posaconazole concentrations are achieved after oral administration because of its weakly basic properties (p*Ka* 3.6 and 4.6) and the acidic environment of the stomach in fasting conditions (pH 1–2). Simulating different intragastric scenarios, literature data clearly indicate that higher gastric concentrations of posaconazole are accompanied by higher systemic exposure.[14-16] Therefore, inter-subject variability in systemic exposure may be attributed to differences in gastric residence time and alternating gastric pH levels between patients, whether or not related to a disease condition or the use of other medication. For instance, coadministration of drugs that reduce acid production (e.g., proton pump inhibitors or H_2-antagonists) resulted in lower systemic exposure of posaconazole because of the elevated pH in the stomach. On the contrary, coadministration of Coca Cola® prolonged gastric residence time, resulting in higher gastric concentrations and improved systemic exposure.[15,16] Despite the beneficial effect of Coca Cola®, however, the oral bioavailability of posaconazole remains limited in fasting conditions.

In view of the fact that absorption takes place in the small intestine, it is obvious that the positive correlation between gastric concentrations and systemic disposition has to be linked to processes taking place upon transfer to the small intestine. One of the possibilities that has been intensively discussed in literature is the creation of supersaturation upon entry in the small intestine, possibly resulting in enhanced drug flux across the intestinal mucosa.[17,18] In general, weakly basic compounds are able to supersaturate in intestinal fluids after gastrointestinal transfer, based on a solubility gradient between stomach (higher solubility) and small intestine (lower solubility). Although supersaturation creates promising perspectives, precipitation of the drug reducing the concentration to its solubility is unavoidable from a thermodynamic point of view.[19,20] Precipitation kinetics, however, may significantly vary depending on physiological variables (e.g., pancreatic and bile secretions, gastrointestinal transfer, hydrodynamics) and physicochemical drug properties (e.g., p*Ka*, solubility, molecular structure). Regarding the fact that low intestinal concentrations were measured after oral administration of the Noxafil® suspension in humans, it was hypothesized that extensive and immediate posaconazole precipitation following gastrointestinal transfer may limit posaconazole absorption.[21]

Although supersaturation/precipitation has been explored intensively *in vitro*, *in vivo* data are rather scarce. Hence, the aim of this study was to investigate the gastrointestinal interplay between dissolution, supersaturation, and precipitation of posaconazole as such in humans. In particular, we evaluated the impact of formulation pH (acidified versus neutral) and formulation type (solution versus suspension) on these processes. To this end, intraluminal concentrations in stomach and duodenum were measured as a function of time following intragastric administration of (i) a neutral suspension of 40 mg posaconazole dispersed at pH 7.1, (ii) an acidified suspension of 40 mg posaconazole dispersed at pH 1.6, and (iii) an acidified solution of 20 mg posaconazole dissolved at pH 1.6. In case of the suspensions, blood samples were collected in parallel in order to study the impact of duodenal supersaturation and precipitation on systemic exposure.

Materials and Methods

Chemicals

Posaconazole was kindly donated by the Chemical Research Division of MSD (Whitehouse Station, New Jersey), whereas itraconazole as such was kindly provided by Janssen Research Foundation (Beerse, Belgium). The marketed suspension of posaconazole, Noxafil® (40 mg/mL), was purchased from the University Hospitals Leuven (Leuven, Belgium). Dimethyl sulfoxide (DMSO) and methanol (MeOH) were received from Acros Organics (Geel, Belgium), whereas BHD Laboratory Supplies (Poole, UK) supplied HCl and NaOH. Acetonitrile and diethylether were purchased from Fisher Scientific (Leicestershire, UK). Sodium acetate and acetic acid were purchased from VWR (Leuven, Belgium). Water was purified using a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

Clinical Study

A cross-over study with two experimental conditions (involving blood sampling) was performed in five healthy volunteers (HVs; three women and two men, aged between 23 and 25 years). Exclusion criteria were checked during a medical examination and included gastrointestinal disorders, infection with hepatitis B, hepatitis C or HIV, use of medication, pregnancy and frequent X-ray exposure. All volunteers provided informed consent to participate in the clinical study. Following the tenets of the Declaration of Helsinki, the clinical study was approved by the Committee of Medical Ethics of the University Hospitals Leuven (ethical approval number ML9945), and the Federal Agency of Health and Medicines (reference number 63285). The study has been saved in the European Clinical Trials Database (EudraCT) with reference number 2013–002836–26. After an overnight fasting period of at least 12 h, volunteers were asked to come to the hospital. A double-lumen polyvinyl catheter (Argyle Salem Sump Tube, 14 Ch [external diameter 4.7 mm]; Sherwood Medical, Tullamore, Ireland) was introduced via the mouth/nose and positioned in the duodenum (D2/D3) of the small intestine. Subsequently, a second double-lumen polyvinyl catheter was positioned in the antrum of the stomach (i.e., lowest part of the stomach). Finally, in order to administer the suspensions of posaconazole intragastrically, a single-lumen polyurethane catheter (Enteral Feeding Tube Wide Bore, 10FR, 100 cm length; Eurosteriel Medical, Dronten, the Netherlands) was positioned in the body of the stomach (i.e., central part of the stomach). Positioning was checked by fluoroscopy (Fig. 1). During the entire experiment, volunteers were sitting in an upright position.

Posaconazole was administered intragastrically as 2 different suspensions:

- an acidified suspension of 40 mg posaconazole at pH 1.6;
- a neutral suspension of 40 mg posaconazole at pH 7.1.

For the acidified suspension, 1 mL of Noxafil® (40 mg posaconazole as such, which corresponds to 10% of the typical therapeutic dose) was dispersed in 240 mL of tap water acidified to pH 1.6 with HCl (70% of posaconazole in solution). For the neutral suspension, 1 mL of Noxafil® (40 mg posaconazole) was dispersed in 240 mL of tap water (pH 7.1; 2.3% of posaconazole in solution).

After intragastric administration, antral and duodenal fluids were aspirated for 3 h; samples were taken at 2, 7, 15, 25, 35, 45, 55, and 60 min during the first hour and every 15 min for the next 2 h. The sampling volume was kept as small as possible (<4 mL per time point). Immediately after aspiration of fluids, pH was measured (Hamilton Knick Portamess®, Bonaduz, Switzerland) and the determination of dissolved and total posaconazole was initiated (see below). Blood samples were collected in heparinized tubes (BD Vacutainer systems, Plymouth, UK) at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, and 24 h after intragastric administration. Blood samples were centrifuged (2880*g*, 10 min, 4°C) and the obtained plasma was stored at −26°C until analysis (see below).

In an additional condition (no blood sampling involved), posaconazole as such was intragastrically administered as a solution in

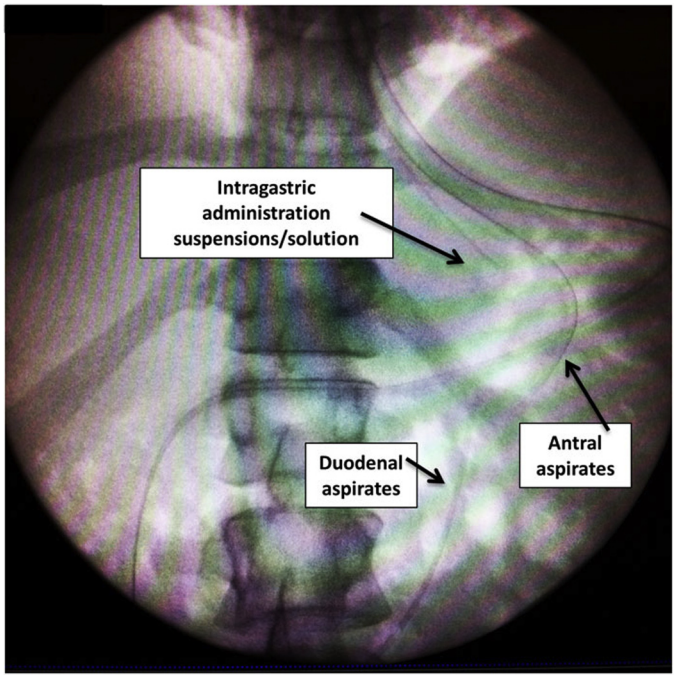

Figure 1. Fluoroscopic image of the position of two double-lumen aspiration catheters and one single-lumen infusion catheter in the gastrointestinal tract of a healthy volunteer.

order to assess intestinal posaconazole precipitation without the potential impact of accelerated nucleation due to the presence of solid particles from the suspensions. The solution was prepared by dissolving 0.5 mL of Noxafil® (20 mg posaconazole) in 240 mL of tap water acidified to pH 1.6. Analogous to previous test conditions, antral and duodenal fluids were aspirated for 3 h, followed by pH measurement (Hamilton Knick Portamess®) and determination of dissolved and total posaconazole concentrations (see below). Blood samples were not collected.

Solubility Experiments

As mentioned in Introduction, a supersaturated solution refers to a state where dissolved concentrations of posaconazole exceed its thermodynamic solubility in the corresponding medium. The degree of supersaturation (DS) is expressed as:

$$DS = \frac{C}{C_{eq}} \quad (1)$$

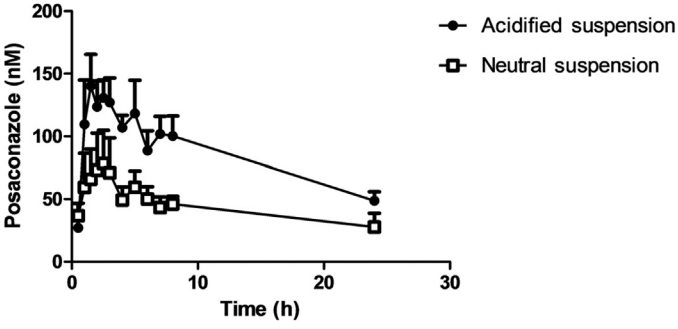

Figure 2. Systemic concentration−time profiles of posaconazole following intragastric administration of the acidified suspension adjusted to pH 1.6 (•) or to neutral suspension adjusted to pH 7.1 (□) (mean + SEM, $n = 5$).

where C stands for the dissolved concentration of posaconazole in the gastric or intestinal fluid at the time of aspiration and C_{eq} stands for the thermodynamic solubility of the drug in the corresponding gastric or intestinal fluid. A solution can be defined as supersaturated, unsaturated, or saturated when DS > 1, DS < 1, or DS = 1, respectively. The thermodynamic solubility of posaconazole was determined in all aspirated gastric or duodenal samples, by adding an excess of posaconazole (20 μL of the 40 mg/mL marketed suspension) to 800 μL of fluid. After 24 h of shaking in a 37°C prewarmed incubator (175 rpm) (KS4000i incubator; Ika, Staufen, Germany), fluids were centrifuged (20,817*g*, 15 min, 37°C). After removing the surface layer of the fluid, the supernatant was diluted 9 times with mobile phase (MeOH:25 mM acetic acid buffer pH 3.5 [85:15, v/v]). Precipitated protein in the samples was separated with an additional centrifugation step (20,817*g*, 5 min, 37°C). Subsequently, 50 μL of the supernatant was injected into the HPLC system and analyzed for posaconazole (see below).

Analysis of Posaconazole in Gastrointestinal Fluids

In order to determine dissolved concentrations of posaconazole in gastric and duodenal fluids, aspirates were immediately centrifuged (20,817*g*, 5 min) and the supernatant was diluted 9 times in mobile phase (MeOH:25 mM acetic acid buffer pH 3.5 [85:15, v/v]). In case of determining total concentrations (i.e., solute + solid) of posaconazole in gastric and duodenal fluids, aspirates were directly diluted ninefold in mobile phase (MeOH:25 mM acetic acid buffer pH 3.5 [85:15, v/v]). Precipitated protein in the samples was separated with an additional centrifugation step (20,817*g*, 5 min, 37°C). Samples were analyzed by HPLC-fluo. In detail, 50 μL of supernatant was injected into the HPLC system consisting of an Alliance as such 2695 separations module and a Novapak C-18 column under radial compression (Waters, Milford, MA); detection was performed by fluorescence (Waters 2475 Multiwavelength Fluorescence Detector) at an excitation wavelength of 240 nm and an emission wavelength of 385 nm. An isocratic run with MeOH:25 mM acetic acid buffer pH 3.5 (85:15, v/v) was performed with a flow rate of 1 mL/min to generate a retention time of 4.3 min. After 6 min, the column was rinsed during 1 min with MeOH:25 mM acetic acid buffer pH 3.5 (75:25, v/v), followed by 2 min with water: 25 mM acetic acid buffer pH 3.5 (75:25, v/v) and subsequently re-equilibrated with the mobile phase for 2 min. Calibration curves were made in mobile phase based on a stock solution of posaconazole as such in DMSO (7 mM). Linearity was observed between 20 μM and 39 nM. The accuracy and precision errors were less than 9% and 6%, respectively, for a concentration of 10 μM in human intestinal fluids collected in the fasted state (FaHIF). Quality control samples of 5, 2.5, and 1.25 μM posaconazole in FaHIF, which were analyzed together with the samples of the *in vivo* study, resulted in a relative SD and accuracy error of less than 8% and 9%, respectively.

Analysis of Posaconazole in Plasma

Analysis of plasma concentrations was performed by extracting posaconazole from the collected samples as described by Walravens et al.[16] Briefly, 100 μL of internal standard solution (2.5 μM itraconazole in 0.2 N HCl) was added to 1000 μL of plasma. Subsequently, the sample was alkalized with 500 μL of 2 N NaOH. After addition of 5 mL diethylether, samples were vortexed for 30 s and directly centrifuged (2880*g*, 10 min, 4°C). Finally, the water layer was discarded and the organic layer was evaporated to dryness under a gentle stream of air. A volume of 300 μl of mobile phase [MeOH:20 mM acetic acid buffer pH 3.3 (76:24, v/v)] was added to the remaining residue. After centrifugation (2880*g*, 1 min, 4°C), 50 μL of the supernatant was injected into the Hitachi Elite LaChrom HPLC

Table 1
Mean Descriptive Parameters of the Gastric, Duodenal, and Systemic Concentration–Time Profiles Following Intragastric Administration of 40 mg Posaconazole as an Acidified Suspension and a Neutral Suspension to Five Healthy Volunteers (Mean ± SD)

Variable	Acidified Suspension (pH 1.6)	Neutral Suspension (pH 7.1)
Plasma		
$AUC_{0\text{-}8\ h}$ (nM h)*	820 ± 284	418 ± 257
C_{max} (nM)*	151 ± 46.3	89.9 ± 59.1
t_{max} (h)	2.80 ± 1.30	3.5 ± 1.3
Antrum		
AUC0-120 min (μM min)	3638 ± 2222	2127 ± 1395
C_{max} (μM)	98.2 ± 63.9	76.9 ± 47.4
t_{max} (min)	19.8 ± 12.3	20.4 ± 12.5
Duodenum		
$AUC_{0\text{-}120\ min}$ (μM min)	892 ± 326	463 ± 201
C_{max} (μM)	26.3 ± 10.3	13.6 ± 5.8
t_{max} (min)	20.2 ± 21	29 ± 16.8

* The observed difference between both suspensions is statistically significant ($p < 0.05$).

system (VWR) and analyzed by an L-2480 fluorescence detector (excitation wavelength 240 nm, emission wavelength 385 nm). A gradient run of 19 min was performed in order to obtain retention times of 7.9 and 12 min on the Novapak C-18 column for posaconazole and itraconazole, respectively. A calibration curve in plasma was made based on stock solutions of posaconazole and itraconazole in DMSO. Linearity was observed between 2000 nM and 7.8 nM. Quality control samples of 500 and 50 nM, which were analyzed together with the plasma samples, resulted in an accuracy and precision error of less than 9% and 8%, respectively.

Data Presentation and Statistical Analysis

Intraluminal concentration–time profiles are presented as mean + standard error of the mean (SEM) for five subjects. Pharmacokinetic parameters (plasma and duodenal C_{max} t_{max}, and area under the curve [AUC]) are reported as mean ± SD. Test conditions were compared using a paired t-test (after logarithmic transformation of C_{max} and AUC); differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed in GraphPad Prism for Windows (GraphPad Software, San Diego, CA). In order to express the precipitated fraction (π) of posaconazole in the aspirated duodenal samples after intragastric administration of the solution, the following equation was used, as previously reported by Psachoulias et al.[22]:

$$\pi = 1 - \frac{C}{C_t} \quad (2)$$

where C stands for the dissolved concentration of posaconazole in the aspirated sample at a specific time point and C_t stands for the total concentration (i.e., dissolved and precipitated) of posaconazole at that same time point.

Results and Discussion

Intragastric Administration of Posaconazole as an Acidified and a Neutral Suspension

Plasma Concentrations

Figure 2 demonstrates the mean plasma concentration–time profiles of posaconazole for all five volunteers after intragastric administration of the acidified and neutral suspensions at pH 1.6 and pH 7.1, respectively. Considering that a similar dose (40 mg of posaconazole) was administered in both conditions, a large difference in systemic exposure could be observed. Compared with the neutral suspension, administration of the acidified suspension resulted in a twofold higher plasma $AUC_{0\text{-}8\ h}$ (819.9 ± 283.5 nM h versus 417.6 ± 258.1 nM h; $p < 0.05$) (Table 1). As a matter of fact, the acidified suspension resulted in a higher plasma $AUC_{0\text{-}8\ h}$ for each single volunteer. A significant twofold increase was also observed for the average plasma C_{max} (151.1 ± 46.3 nM for the acidified suspension versus 89.9 ± 59.1 nM for the neutral suspension; $p < 0.05$) (Table 1). Although the mean plasma t_{max} did not significantly differ between both conditions (2.8 ± 1.3 h for the acidified suspension versus 3.5 ± 1.3 h for the neutral suspension; $p > 0.05$), a lower t_{max} could be observed for three out of five volunteers following administration of the acidified suspension. In summary, the increased $AUC_{0\text{-}8\ h}$ and C_{max}, and the decreased t_{max} indicate improved intestinal uptake of posaconazole administered at pH 1.6. Remarkably, the observed average C_{max} after administration of the acidified suspension in the present study appeared similar to the mean C_{max} reported in a previous study (155 ± 46 nM)[16] after oral administration of a 10-fold higher therapeutic dose (i.e., 400 mg posaconazole) as a suspension. In order to understand the observed systemic exposure of posaconazole, its behavior was investigated at the level of the gastrointestinal lumen where dissolution, precipitation, and absorption take place.

Gastric Concentrations

After intragastric administration of the acidified suspension at pH 1.6 (A) or the neutral suspension at pH 7.1 (B), dissolved and

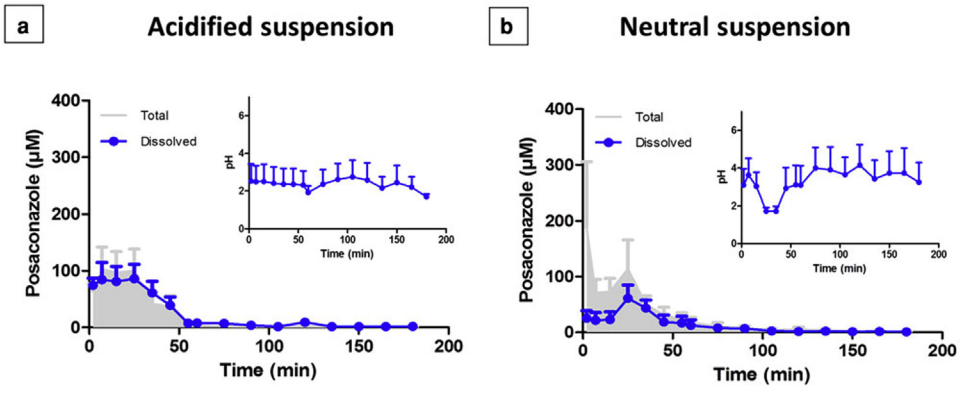

Figure 3. Gastric concentration–time profiles for dissolved (•) and total (gray curve) concentrations of posaconazole after intragastric administration of (a) the acidified suspension (pH 1.6) and (b) the neutral suspension (pH 7.1). The upper right inserts show the pH of the gastric aspirates as a function of time (mean + SEM, $n = 5$).

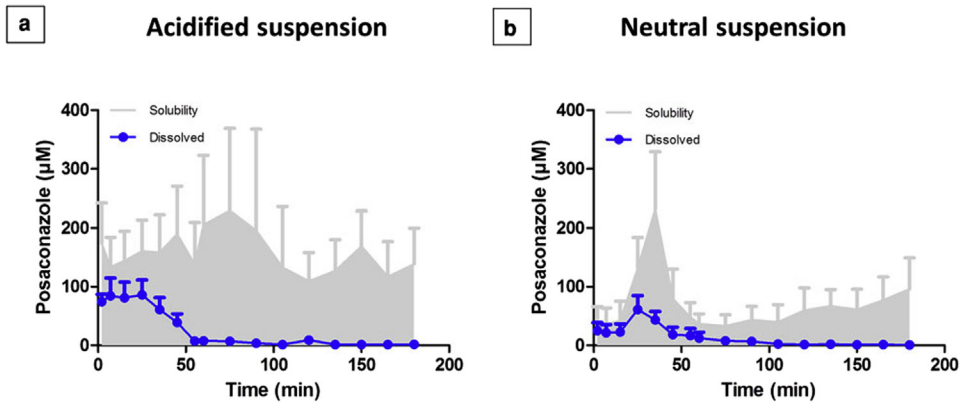

Figure 4. Gastric concentration–time profiles for dissolved concentrations (•) and solubility (gray curve) of posaconazole after intragastric administration of (a) the acidified suspension (pH 1.6) and (b) the neutral suspension (pH 7.1) (mean + SEM, $n = 5$).

total concentrations of posaconazole were monitored in the antrum (Fig. 3). On average, a high gastric C_{max} of 98.2 ± 63.9: µM was observed after administration of the acidified suspension as a result of (i) the predissolved state of most posaconazole in the acidified suspension and (ii) the acidic pH of the gastric fluids in fasting conditions (Fig. 3a). Based on the ratio of the AUC of the dissolved versus total (i.e., solute + solid) concentration profiles, posaconazole as such was almost completely dissolved in the stomach after intragastric administration of the acidified suspension (94 ± 73%; Fig. 3a).

In contrast, administration of the neutral suspension resulted in a more distinct difference between total and dissolved concentrations, indicating the presence of more undissolved posaconazole in the stomach (Fig. 3b). As can be extracted from Figure 4b, however, the solubilizing capacity of the aspirated gastric fluids for posaconazole as such exceeded dissolved concentrations at the time of aspiration (subsaturation). The extent of posaconazole dissolution in the stomach most likely depends on the gastric motility and residual volume, affecting the dissolution rate. Fast gastric emptying may therefore result in incomplete dissolution of posaconazole in these fluids. Interestingly, a reduction in gastric pH resulted in increased posaconazole solubility and concentration (as reflected in the mean profiles on Figs. 3b and 4b). Although antral concentrations of posaconazole were higher for the acidified suspension than for the neutral suspension in each single volunteer, the differences in mean antral $AUC_{0\text{-}120\ min}$ or C_{max} were not statistically significant ($p > 0.05$) (Table 1).

Intestinal Concentrations

After gastrointestinal transfer, the physiological change in pH, fluid composition, and fluid volume may dramatically influence the subsequent intestinal behavior of a drug.[23] For poorly soluble weakly basic drugs, this transfer may be accompanied with a solubility drop and the induction of supersaturation. Considering the antral concentrations of posaconazole following administration of both suspensions (up to 100 µM; Fig. 3), and the reported low solubility in duodenal fluids (ca. 5 µM),[24] supersaturation of posaconazole as such is likely for both conditions upon entry in the small intestine.

As indicated by the arrows in Figure 5a, total posaconazole clearly exceeds dissolved posaconazole in the duodenal aspirates after intragastric administration of the acidified suspension. Although solid posaconazole may partly consist of particles transferred from the stomach, the large difference in dissolved fraction between stomach (Fig. 3a) and small intestine (Fig. 5a) clearly suggests intestinal precipitation. Despite precipitation, mean posaconazole as such concentrations still exceeded the average solubilizing capacity of the aspirated intestinal fluids for about 45 min following administration of the acidified suspension (Fig. 6a). When comparing the posaconazole concentration in individual samples to the corresponding solubility, limited supersaturation was indeed observed, as indicated in the insert of Figure 6a.

In comparison, the neutral suspension generated lower, (sub) saturated duodenal concentrations of posaconazole, most likely resulting from immediate precipitation and/or dilution upon entry

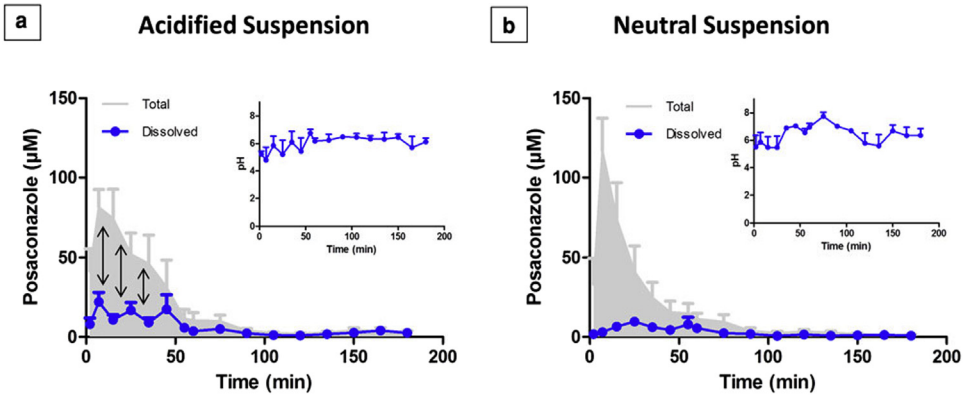

Figure 5. Duodenal concentration–time profiles for dissolved (•) and total (gray curve) concentrations of posaconazole after intragastric administration of (a) the acidified suspension (pH 1.6) and (b) the neutral suspension (pH 7.1). The upper right insert shows the pH of the duodenal aspirates as a function of time (mean + SEM, $n = 5$).

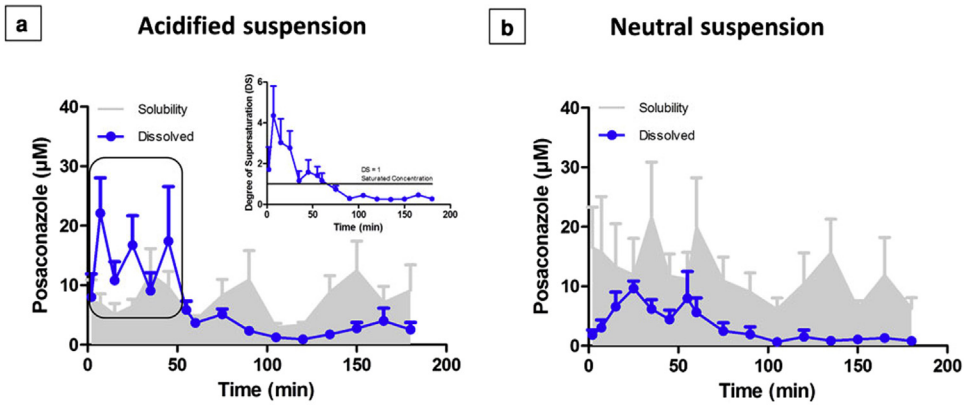

Figure 6. Duodenal concentration–time profiles for dissolved concentrations (•) and solubility (gray curve) of posaconazole after intragastric administration of (a) the acidified suspension (pH 1.6) and (b) the neutral suspension (pH 7.1). The upper right insert in Figure 6a shows the degree of supersaturation (DS) as a function of time, determined by the ratio of posaconazole concentration and solubility in individual fluid samples. The time window of supersaturated posaconazole concentrations is indicated by the black box (mean + SEM, $n = 5$).

in the small intestine (Fig. 6b). The fraction of solid posaconazole in the intestinal aspirates was even higher than for the other experimental condition (Fig. 5b).

Regarding the acidified suspension, the observed posaconazole supersaturation and consequently increased duodenal concentrations apparently resulted in a faster flux of posaconazole across the intestinal membrane. Indeed, the approximately twofold higher duodenal $AUC_{0\text{-}120\ min}$ and C_{max} values (Table 1) were reflected in the aforementioned systemic profiles (higher plasma AUC and C_{max} but decreased t_{max} for the acidified suspension in comparison with the neutral suspension). Thus, even though only a low DS was observed, it was still beneficial for intestinal absorption compared with the neutral suspension. When considering the total concentrations of posaconazole, a mean duodenal t_{max} of 7 min was observed in both conditions (Fig. 5). Interestingly, the fast appearance of posaconazole in the small intestine is in the same line with recently published data regarding the gastrointestinal transfer.[25]

The results of the present study clearly identify intestinal precipitation of posaconazole as one of the key issues causing limited and variable fasted state absorption, in addition to the previously reported incomplete and pH-dependent gastric dissolution.[16,26] In this respect, it is worth mentioning that a novel delayed-release tablet of posaconazole has recently been approved by the United States Food and Drug Administration (November 2013). The tablet contains 100 mg of posaconazole dispersed in a matrix of hydroxypropyl methylcellulose acetate succinate (HPMC-AS) that only releases posaconazole in the neutral environment of the intestine, thereby circumventing the variable gastric dissolution.[27-30] Assuming that fast release from this solid dispersion enables increased intestinal concentrations of posaconazole, the polymer HPMC-AS may stabilize possible supersaturation by acting as a precipitation inhibitor.[31-33]

Intragastric Administration of Posaconazole as an Acidified Solution: Comparison With Literature Data on the Intestinal Precipitation of Weak Bases

Our results suggest significant intestinal precipitation of posaconazole as such following intragastric administration of the suspensions. It should be noted, however, that gastrointestinal transfer of even a few undissolved particles may have accelerated precipitation of posaconazole in the duodenum. In order to circumvent this complicating factor in evaluating the precipitation kinetics of posaconazole, a solution of posaconazole (20 mg at pH 1.6) was intragastrically administered to the HVs as a third test condition. Dissolved and total gastric posaconazole concentrations are shown as a function of time in Figure 7a. In all volunteers, posaconazole remained in solution in the gastric fluids, prior to transfer to the small intestine. Regarding the intraluminal behavior of posaconazole upon entry in the duodenum, Figure 7b depicts a clear difference between the dissolved and total duodenal concentrations of posaconazole, illustrating extensive precipitation as

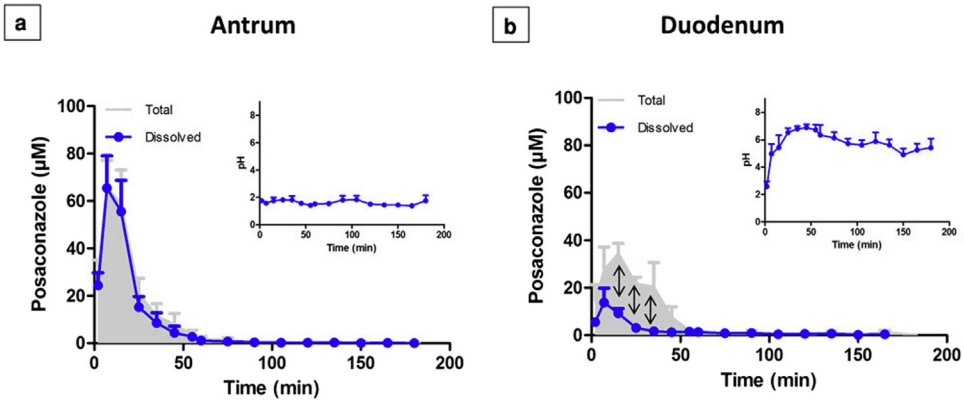

Figure 7. Antral (a) and duodenal (b) concentration–time profiles for dissolved (•) and total (gray curve) concentrations of posaconazole after intragastric administration of the solution. The upper right figures show the pH of the antral and duodenal aspirates as a function of time (mean + SEM, $n = 5$).

Table 2
Degree of Supersaturation (DS; Mean ± SD) and Precipitated Fraction (π; Mean ± SD) of Posaconazole in the Upper Small Intestine as a Function of Time for the First 60 Min After Intragastric Administration of the 20 mg Posaconazole Solution

Time (min)	Degree of Supersaturation (DS)	Precipitated Fraction (π)
2	0.23 ± 0.24	0.62 ± 1.81
7	2.9 ± 14	0.50 ± 0.85
15	1.3 ± 3.4	0.73 ± 1.2
25	0.79 ± 1.7	0.86 ± 1.25
35	0.58 ± 1.1	0.92 ± 2.1
45	0.47 ± 0.60	0.83 ± 1.99
55	0.23 ± 0.60	0.22 ± 0.09
60	0.56 ± 1.3	0.05 ± 0.04

indicated by the arrows. As reported in Table 2, mean precipitated fractions up to 92% were observed.

In Figure 8, the maximum precipitated fraction observed for posaconazole is compared with reported values for two other weakly basic drugs, ketoconazole and dipyridamole. Similar as in this present study, acidified solutions of ketoconazole or dipyridamole as such were administered intragastrically in HVs.[22] After administration, duodenal contents were supersaturated up to maximum 50 min and precipitation was limited (≤7% for dipyridamole, ≤16% for ketoconazole). In comparison, significantly higher precipitation of posaconazole was observed in the present study (up to 92%), despite a minimal and short-term period of supersaturation (Table 2). DS and precipitated fraction (π) were calculated based on Equations 1 and 2, respectively. Based on physiology-based sensitivity analysis, extensive and immediate intestinal precipitation of posaconazole was already anticipated to explain the low duodenal concentrations observed in humans following intake of a therapeutic dose (400 mg posaconazole) of the oral suspension.[16,21] It was hypothesized that undissolved posaconazole particles from the suspension accelerated posaconazole precipitation by acting as nuclei for crystal growth. The current study indicates that also in the absence of nuclei (posaconazole solution), the tendency for precipitation is higher for posaconazole than for ketoconazole and dipyridamole.

Shono et al.[34] used *in vitro* dissolution data of the mesylate salt of another weak base, nelfinavir, as input for physiologically based pharmacokinetic modeling in order to predict the *in vivo* plasma concentration–time profiles of the drug. Only when including the possibility of significant precipitation (precipitated fraction about 50%) in the model, simulated plasma profiles mimicked the performance of nelfinavir in fasting conditions. Hence, when comparing human precipitation data for different weak bases, it is clear that, apart from the physiological variables of the gastrointestinal tract (e.g., motility, transfer, dilutions, and intestinal uptake), the physicochemical properties of the compound will have a major influence on the precipitation kinetics of the drug upon entry in the small intestine.[19]

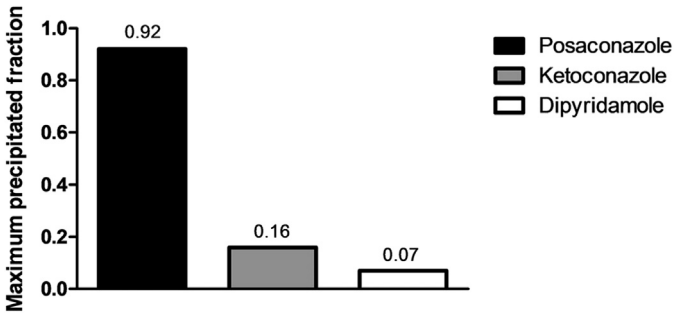

Figure 8. Maximum precipitated fraction of posaconazole in comparison with the reported maximum precipitated fraction of ketoconazole and dipyridamole[22] after intragastric administration of drug solutions.

Conclusions

This study explored the intraluminal dissolution, supersaturation, and precipitation behavior of the weak base posaconazole after intragastric administration of two suspensions and a solution of posaconazole to humans. After administration of the suspensions, fasted-state absorption of posaconazole was hampered by incomplete gastric dissolution (in case of the neutral suspension) and/or significant intestinal precipitation. Despite precipitation, however, limited intestinal supersaturation of posaconazole following administration of the acidified suspension (pH 1.6) resulted in increased absorption as compared with the neutral suspension (pH 7.1). Extensive precipitation of posaconazole was also observed after administration of a solution, contrasting with previously published data for two other basic compounds. This indicates that precipitation kinetics *in vivo* are strongly affected by the physicochemical properties of the compound itself, in addition to physiological variables. In a next step, these data will serve as unique reference data for the optimization and validation of *in vitro* and *in silico* simulation tools for supersaturation and precipitation, as described in the European IMI project, OrBiTo.[35,36]

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