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Research paper

Gastrointestinal dissolution, supersaturation and precipitation of the weak base indinavir in healthy volunteers

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ABSTRACT

This study investigated the impact of relevant gastrointestinal conditions on the intraluminal dissolution, supersaturation and precipitation behavior of the weakly basic drug indinavir. The influence of (i) concomitant PPI intake and (ii) the nutritional state on the gastrointestinal behavior of indinavir was assessed in order to identify the underlying mechanisms responsible for previously reported interactions. Five healthy volunteers were recruited into a crossover study containing the following arms: fasted state, fed state and fasted state with concomitant proton pump inhibitor (PPI) use. In each condition, one Crixivan® capsule (400 mg indinavir) was orally administered with 240 mL of water. Gastric and duodenal fluids, aspirated as a function of time, were monitored for total and dissolved indinavir concentrations on a UPLC-MS/MS system. Indinavir's thermodynamic solubility was determined in individual aspirates to evaluate supersaturation. The bioaccessible fraction of indinavir in aspirated duodenal fluids was determined in an *ex vivo* permeation experiment through an artificial membrane.

A nearly complete dissolution of indinavir in the fasted stomach was observed (90 ± 3%). Regardless of dosing conditions, less indinavir was in solution in the duodenum compared to the stomach. Duodenal supersaturation was observed in all three testing conditions. The highest degrees of duodenal supersaturation (6.5 ± 5.9) were observed in the fasted state. Concomitant PPI use resulted in an increased gastric pH and a smaller fraction of indinavir being dissolved (58 ± 24%), eventually resulting in lower intestinal concentrations. In fed state conditions, drug release from the capsule was delayed and more gradually, although a similar fraction of the intragastric indinavir dissolved compared to the fasted state (83 ± 12%). Indinavir was still present in the lumen of the duodenum three hours after oral administration, although it already reached 70% (on average) of the fasted state concentrations (expressed as AUC_{0-3} h). Based on a 2-h permeation experiment, the bioaccessible fraction of indinavir was 2.6-fold lower in a fed state sample compared to a fasted state sample.

Our data indicate that the reported reduction in indinavir's bioavailability after concomitant PPI administration is caused by an elevated gastric pH resulting in less indinavir in solution in the stomach and, subsequently, reduced duodenal concentrations. In fed state conditions, however, intestinal micellar entrapment of indinavir appeared to cause the reported reduced bioavailability, regardless of duodenal concentrations.

1. Introduction

Since the 1960s, more and more drug candidates suffer from poor oral bioavailability due to low water-solubility [1]. To overcome this challenge, formulation strategies have been developed to (temporarily) increase drug concentrations and thus increase oral bioavailability. These strategies include the use of salts, cocrystals, solid dispersions and self microemulsifying systems [2]. After oral ingestion, these formulations pass through the complex environment of the gastrointestinal tract. The pharmacokinetic performance of drugs formulated in this way can be substantially influenced by the gastrointestinal environment (motility, transit, intraluminal fluid composition); however, the underlying mechanisms are often insufficiently understood [3]. Furthermore, current *in vitro* tools fail to capture the influence of

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the complex gastrointestinal environment on these absorption-enabling formulation strategies, making it difficult to predict their performance [4]. In order to expand our knowledge on the *in vivo* gastrointestinal behavior of a drug, an intraluminal sampling method has been developed to monitor gastric and duodenal drug concentrations over time [3].

Certain low aqueous solubility drugs can temporarily reach intraluminal concentrations exceeding their equilibrium solubility. Weakly basic drugs, for example, can supersaturate when transferred from an environment with high solubility (e.g. acidic stomach) to one with low solubility (e.g. neutral intestine) [5]. The temporarily increased concentrations in the small intestine may imply an improved driving force for absorption and result in enhanced bioavailability [6]. Since supersaturation is a thermodynamically unstable state, precipitation of the drug to its equilibrium solubility is inevitable and may limit the beneficial effect. Recent studies have confirmed the ability of weakly basic drugs to become supersaturated *in vivo*. Using the aforementioned intraluminal sampling method, intestinal supersaturation of dipyridamole, ketoconazole and posaconazole upon gastric emptying was observed [7,6]. Furthermore, intestinal supersaturation of abiraterone following hydrolysis of the ester prodrug abiraterone acetate was observed [8].

The aim of the present study was to investigate the gastrointestinal behavior of the protease inhibitor indinavir. Indinavir is a weak base with pKa's of 3.7 and 5.9. The solubility of indinavir is very high in an acidic environment (>162 mM at pH < 3.5) but relatively low in a neutral environment (0.05 mM at pH 6) [9]. Since the intestinal permeability for indinavir sits on the borders of the BCS Classes [10], indinavir has been reported to be either a BCS class II (low solubility, high permeability) or a BCS class IV (low solubility, low permeability) drug [11–15]. The intestinal behavior of indinavir can be complicated as the average duodenal pH varies (5.6–7.0, median 6.3) around the pKa of indinavir [16]. Small fluctuations in pH may thus imply large fluctuations in solubility. In the right conditions, supersaturation could occur when indinavir is transferred from stomach to intestine. In addition, indinavir is marketed as the sulfate salt (Crixivan®, 400 mg capsules). In animal and human studies, the sulfate salt has superior oral pharmacokinetics compared to the free base. While the free base is only adequately absorbed when administered as an acidic solution, the sulfate salt can be administered as a solid dose to obtain sufficient plasma concentrations [17]. Drugs formulated as crystalline salts can generate supersaturation upon dissolution [5]; intake of the crystalline sulfate salt of indinavir may therefore result in the creation of a supersaturated indinavir solution. Most likely, however, this only occurs in a neutral environment considering the pH-dependent solubility of indinavir.

Furthermore, Tappouni et al. observed an interaction between the proton pump inhibitors (PPI) omeprazole and indinavir; co-administration resulted in a lower systemic exposure (AUC_{0-24h} −47%) This was explained by an increase in intragastric pH causing a drastically lower solubility of the drug and less driving force for intestinal supersaturation and absorption [18]. Similar observations were made in a retrospective study comparing population pharmacokinetic parameters with individuals on a PPI regime [19].

In contrast to many poorly soluble protease inhibitors that show a positive food effect (increase in AUC), indinavir's bioavailability is negatively affected by intake of a high-fat meal (decrease in AUC) [20–22]. It has been suggested that this effect could be attributed to a delayed gastric emptying in combination with precipitation of indinavir due to a higher gastric pH and/or a decreased absorption due to micellar entrapment in the intestine [22,11].

Overall, indinavir's pH-dependent solubility and formulation strategy suggest an important effect of different physiological intraluminal processes on its absorption *in vivo*. Furthermore, a clear influence of different gastrointestinal conditions (concomitant PPI use and fed state) on the oral bioavailability of indinavir has been established. While explanations are being sought in the intraluminal behavior of indinavir, this has not been thoroughly investigated *in vivo* yet.

This study investigated the intraluminal dissolution, supersaturation and precipitation behavior of the weakly basic drug indinavir. Intragastric and intraduodenal drug concentrations were monitored as a function of time following oral administration of the drug with 240 mL of water. The influence of (i) concomitant PPI intake and (ii) the nutritional state on the gastrointestinal behavior of indinavir was assessed to identify the underlying mechanisms responsible for the altered bioavailability.

2. Materials and methods

2.1. Chemicals

Indinavir sulfate was donated by Hetero Drugs Ltd. (Hyderabad, India). The marketed capsule of indinavir (Crixivan®, 400 mg as sulfate salt, Merck, New Jersey, USA) and the PPI esomeprazole (Nexiam®, 40 mg, AstraZeneca, London, UK) were purchased from the University Hospitals Leuven (Leuven, Belgium). Methanol and formic acid were purchased from Biosolve (Valkenswaard, The Netherlands). Dimethyl sulfoxide (DMSO) was purchased from Acros Organics (Geel, Belgium). Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

2.2. Clinical study

Five healthy volunteers (HVs) (one male and four females) were recruited into a crossover study with the following conditions: (i) fasted state, (ii) fed state and (iii) fasted state with concomitant PPI use. The study obeyed the tenets of the Declaration of Helsinki and Tokyo. The study was registered in the European Clinical Trials Database (EudraCT, 2014-002830-30) and approved by the Federal Agency of Health and Medicines (FAHMP, 750788) and by the Committee of Medical Ethics of the University Hospitals Leuven (ML11279). Exclusion criteria were (a history of) gastrointestinal disorders, use of medication, pregnancy and infection with hepatitis B, C or HIV. Prior to the study, all volunteers provided written informed consent. HVs were fasted for at least 12 h prior to testing. On the first day of the study, female volunteers were checked for pregnancy prior to participation. Volunteers were intubated through the mouth or nose with two double-lumen polyvinyl catheters [Argyle Salem Sump Tube, 14 Ch (4.7 mm × 108 cm); Covidien, Dublin, Ireland]. One catheter was positioned in the antrum of the stomach, and another in the duodenum. The position of the catheters was checked using X-ray fluoroscopy. Subsequently, volunteers were seated in a hospital bed for the duration of the trial. One Crixivan® capsule was administrated orally with 240 mL of tap water in all three conditions. The fed state condition was simulated by administrating 400 mL of Ensure® Plus (Vanilla flavor; Abbott Nutrition, Zwolle, The Netherlands) 20 min prior to Crixivan® administration. For the PPI condition, volunteers were asked to follow a regime of one Nexiam® tablet a day, starting 2 days prior to the study and taking the last tablet the morning of the study. Following Crixivan® ingestion, gastric and duodenal fluids were aspirated through the catheters with the help of 50 mL catheter tip syringes (Terumo Europe, Leuven, Belgium). The sampling volume was kept to a minimum (<3 mL) in an attempt to minimize the amount of indinavir removed through aspiration. Samples were taken at fixed time points: 2, 7, 12, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135, 150, 165, and 180 min. Immediately after aspiration, the pH was measured

(Hamilton Knick Portamess®, Bonaduz, Switzerland) and samples were prepared for indinavir analysis.

2.3. Sample preparation

Aliquots of the aspirated gastric and duodenal samples were diluted 100-fold in methanol:water (50:50, v/v) to assess total indinavir content (solid + solute). Subsequently, aliquots of the same aspirates were centrifuged (20,817*g*, 5 min; microcentrifuge 5424; VWR International) to separate solid from dissolved indinavir. The supernatant was diluted 100-fold in methanol:water (50:50, v/v) to assess the dissolved indinavir concentration. After this sample preparation, the remaining aspirated fluids were stored on ice until transfer from hospital to laboratory. In the laboratory, aspirated samples were stored at −26 °C.

2.4. Assessment of solubility and supersaturation

In order to evaluate indinavir supersaturation, the dissolved indinavir concentration was compared to the equilibrium solubility of indinavir in all individual duodenal aspirates and in the gastric aspirates from the PPI condition. The thermodynamic solubility was determined using the shake-flask method [23]. An excess amount of indinavir sulfate powder was added to a microcentrifuge tube containing 300 μL of duodenal aspirate. These tubes were placed for 24 h in a shake incubator (KS4000i incubator; Ika, Staufen, Germany) at 37 °C and 175 rpm allowing indinavir to reach thermodynamic solubility. A preliminary test demonstrated that 24 h was needed to reach the equilibrium solubility (data not shown). Subsequently, samples were centrifuged (20,817*g*, 15 min, 37 °C) separating undissolved indinavir powder. The supernatant was diluted 100-fold in methanol: water (50:50, v/v) to determine indinavir solubility. The degree of supersaturation (DS) was calculated as follows:

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

C_{eq} stands for the thermodynamic solubility of indinavir in the intestinal fluid and C stands for the dissolved indinavir concentration in the corresponding aspirate. Indinavir is supersaturated if DS > 1.

2.5. Assessment of bioaccessible fraction

Two duodenal samples from HV 04 (one fasted and one fed) with similar indinavir concentrations (711 and 689 μM after the elimination of solid particles, respectively) were selected in order to compare the bioaccessible fraction in fasted and fed state conditions. For this purpose, indinavir permeation from these aspirates was assessed across cellulose membrane strips [pore size ±3 nm, impermeable for micelles with diameter ± 10 nm [24]] in a HTD 96b from HTDialysis, LLC (Gales Ferry, CT, US). Donor compartments were filled with 150 μL of intestinal fluids. Acceptor compartments were filled with 150 μL of phosphate buffer with osmolality and pH equal to the corresponding donor compartment. Osmolality was measured with an Advanced Instruments osmometer model 3250 (Norwood, MA, USA); pH was measured with the instrumentation mentioned above. Samples were taken from the acceptor compartment after 1, 2 and 6 h. The experiment was performed at room temperature. Samples were diluted 10-fold in methanol:water (50:50, v/v) prior to indinavir quantification.

2.6. Indinavir analysis

All sample analyses were performed using an Acquity H-class UPLC system (Waters, Milford, MA, USA) and a Kinetex C18 column (50 × 3 mm, 1.7 μm; Phenomenex) column held at 35 °C (Waters, Milford, MA, USA). The injection volume was 0.1 μL. Methanol (solvent A) and 0.05% formic acid in water (solvent B) were used as eluent at 500 μL/min. Gradient elution was performed as follows: 50% A for 0.3 min, from 50% to 100% A in 0.71 min, and 100% A for 0.30 min. Prior to the next injection, the column was equilibrated with 50% A for 1 min. Detection was carried out on a XEVO-TQS micro triple quadrupole mass spectrometer equipped with an ESI source (Waters, Milford, MA, USA). The mass spectrometer was operated in MS/MS positive ionization mode with following parameters: 150 °C source temperature, 0.75 kV capillary voltage, 12 V cone voltage, 50 L N_2/h cone flow, 1000 L N_2/h desolvation gas flow, 500 °C desolvation temperature The ion transfer used to detect indinavir was from *m*/*z* 614.37 to *m*/*z* 421.20 (Collision energy: 31 V) with a scan time of 59 ms. A divert valve was used to lead the UPLC eluent into the MS within the time frame of 0.3–1.6 min and into the waste for the remaining time. Stock solutions of indinavir (sulfate) were prepared in DMSO and calibration curves were made daily by serial dilution in 50:50 methanol: water. Calibration curves were linear over the range of 50–0.20 μM. For validation, representative fasted state human gastric fluids (FaHGF) and fed state human gastric fluids (FeHGF) were spiked with 2000, 500 and 20 μM indinavir. Following a 1:100 (v/v) dilution, indinavir in gastric samples could be determined accurately (range 96–106%) and precisely (highest intraday variability RSD 4.5% (n = 5), highest interday variability RSD 4.0% (n = 5)). In addition, fasted state human intestinal fluids (FaHIF) and fed state human intestinal fluids (FeHIF) were spiked with 200 and 20 μM indinavir. Also in intestinal samples, indinavir could be determined accurately (range 97–103%) and precisely (highest intraday variability RSD 3.2% (n = 5), highest interday variability RSD 6.4% (n = 5)). During each run with samples from the clinical trials, two quality control samples of 22 μM and 5 μM in 50:50 methanol:water were analyzed, resulting in accuracy errors and RSD of less than 10% and 3.2%, respectively.

2.7. Data presentation and calculations

Mean (+S.E.M.) gastric and duodenal concentration-time profiles of five HVs are presented with inserts depicting the corresponding mean (+S.E.M.) pH-time profile. The duodenal DS was calculated at each sampling point for each HV using Eq. (1). The mean (+S.E.M.) duodenal DS-time profile is presented with inserts of the corresponding concentration/solubility-time profile. The AUC_{0-3h} of indinavir in solution was calculated using the linear trapezoidal method and is presented as mean (+S.E.M.). Percentages dissolved were calculated as the ratio of the AUC_{0-3h} of dissolved indinavir to the AUC_{0-3h} of total indinavir content and are presented as mean (+S.E.M.).

3. Results and discussion

3.1. Fasted state versus fasted with concomitant ppi use

Fig. 1 depicts mean gastrointestinal concentration-time profiles of indinavir following administration of Crixivan® in two test conditions (fasted state and fasted state with concomitant PPI use). The fasted state gastric profile (Fig. 1a) depicts a nearly perfect overlay of dissolved and total indinavir concentrations indicating that almost all indinavir released is in solution. On average 90% of the observed intragastric indinavir is in solution (Fig. 2a). Most

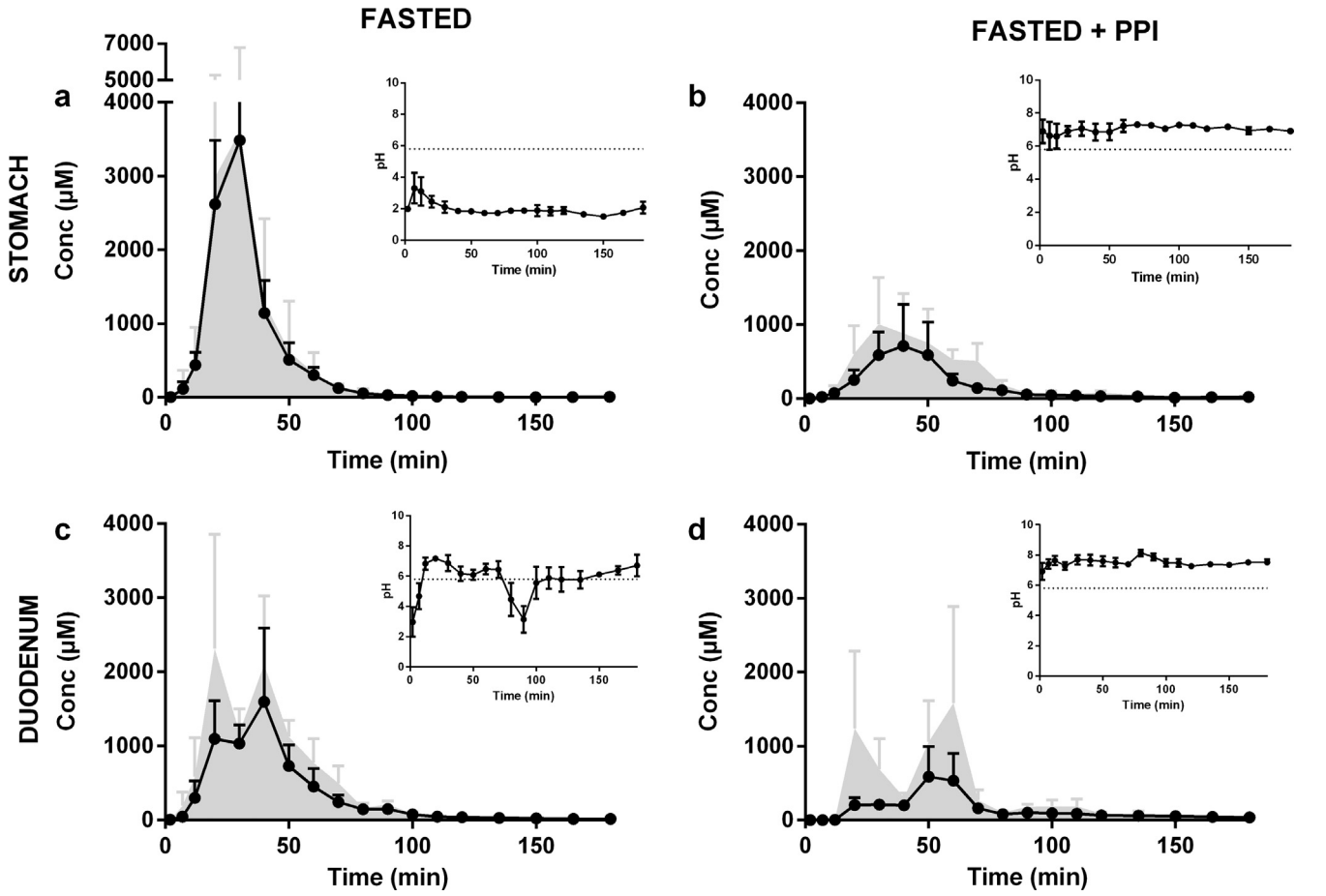

Fig. 1. Gastric and duodenal concentration-time profiles for indinavir following administration of Crixivan® in fasted state (a, c) and fasted state with concomitant use of the PPI esomeprazole (b, d). One capsule of Crixivan® was orally ingested with 240 mL of tap water. Black lines and gray areas represent the dissolved and total Indinavir content (solid + solute expressed as concentration), respectively. Inserts depict the corresponding pH profiles as a function of time. The dashed line represents the pKa of indinavir (5.8). (a) Fasted state, stomach, (b) Fasted state with concomitant PPI use, stomach, (c) Fasted state, duodenum, (d) Fasted state with concomitant PPI use, duodenum (mean ± S.E.M., n = 5).

likely, this is the result of the fast dissolution of the sulfate salt of indinavir and the adequate solubility of the weak base (pKa = 5.8) in the acidic environment of a fasted stomach [insert Fig. 1a; mean pH = 2.02, SD ± 0.89 (n = 89)]. In one HV, the co-administrated 240 mL of tap water (pH = 7.1) resulted in a temporary pH increase above the pKa of indinavir [up to 6.9 (data not shown)]. However, this had no influence on the overall intragastric indinavir concentration-time profile and the percentage of drug dissolved as the pH returned to basal acidity within 20 min, i.e. before relatively large indinavir concentrations were reached. These low concentrations could be due to a high intragastric volume (cfr. 240 mL of water ingested) and/or incomplete release of indinavir from the formulation at these early time points. Due to indinavir's excellent solubility in the acidic environment (>162 mM at pH < 3.5), no supersaturation can be generated in a fasted stomach [9]. When a Crixivan® capsule is co-administrated with a PPI, lower total indinavir concentrations are observed in the stomach compared to the fasted state (Fig. 1b). PPIs inhibit gastric acid secretion by binding to the proton pump (H^+, K^+ -ATPase) [25]. This causes an elevated gastric pH as seen in the insert in Fig. 1b [mean gastric pH 7.18, SD 0.96 (n = 87)]. The elevated pH seems to influence indinavir release from the capsule as reflected by the lower total concentrations; slower drug release from the capsule at elevated pH was also confirmed *in vitro* (data not shown). Furthermore, concomitant PPI use resulted in less similarity between total and dissolved indinavir in the stomach (Fig. 1b); on average 58% of indinavir appeared in solution [compared to 90% in the absence of PPI (Fig. 1a)]. The remaining 42% solid indinavir is a mixture of undissolved particles released from the formulation and precipitated indinavir due to supersaturation (see below). The elevated pH exceeds the pKa of indinavir, implying that dissolved indinavir will be mostly present in its unionized form with decreased solubility. It should be noted, however, that Crixivan® contains the crystalline indinavir sulfate ethanolate form, which dissolves more rapidly than the free base and may generate a higher apparent solubility [17,5]. As a result, the free base indinavir may become supersaturated in a neutral gastric environment. To investigate this assumption, the equilibrium solubility of indinavir was measured in each aspirated sample; supersaturation was revealed by comparing the indinavir concentration in solution with the measured solubility (see Eq. (1)). The mean DS as a function of time is depicted in Fig. 3. Gastric supersaturation was observed in all 5 HVs with a mean maximum DS of 4.3 (SD ± 3.3, n = 5).

Following gastric emptying, indinavir arrives in the duodenum. The solubility of indinavir in this neutral environment [(mean pH = 6.72, SD ± 1.38 (n = 159)] is drastically reduced compared to the solubility in the stomach [9]. The occurrence of a temporary pH drop in the average profile cannot be tied to a certain event. It was observed in two HVs and never coincided with the C_{max}. In the duodenum, larger differences between total and dissolved indinavir are observed compared to the stomach (Fig. 1c and d). For the fasted state an average of 69% indinavir is in solution

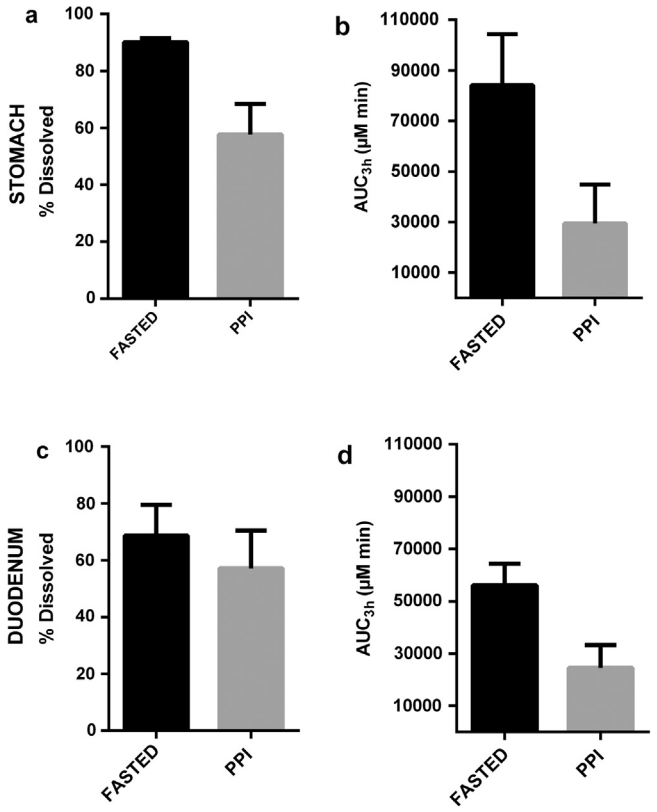

Fig. 2. Dissolved indinavir in stomach and duodenum following administration of Crixivan® in fasted state and fasted state with concomitant use of the PPI esomeprazole. (a) Percentage of indinavir dissolved in the stomach. (b) AUC_{0-3h} of the gastric concentration-time profiles. (c) Percentage of indinavir dissolved in the duodenum. (d) AUC_{0-3h} of the duodenal concentration-time profiles. Percentages dissolved were calculated as the ratio of the AUC_{0-3h} of dissolved indinavir to the AUC_{0-3h} of total indinavir content. Black and gray bars represent fasted and PPI state, respectively (mean ± S.E.M., n = 5).

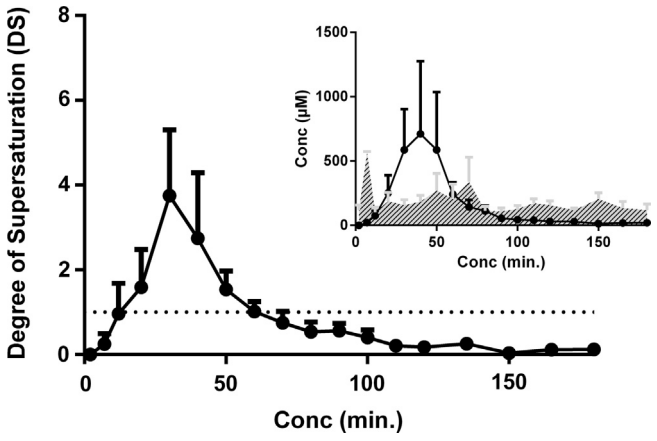

Fig. 3. Degree of indinavir supersaturation (DS) in the stomach as a function of time after oral administration of CRIXIVAN® in fasted state with concomitant use of a PPI. The DS is determined as the ratio of indinavir concentration to the indinavir solubility in each individual fluid sample. The dashed line (DS = 1) represents a saturated concentration. The insert depicts the gastric indinavir concentration (black line) and solubility (banded area) as a function of time (mean ± S.E.M., n = 5).

(Fig 2c). In the fasted state, the non-dissolved particles of indinavir are most likely caused by precipitation of the drug upon transfer as almost all intragastric indinavir was dissolved. In a fasted state with concomitant PPI use, a lower percentage of dissolved indinavir was observed in the duodenum (57% on average, Fig 2c). The remaining non-dissolved indinavir particles are likely a mix of undissolved particles (originating from the formulation) and precipitated indinavir due to gastric and duodenal supersaturation. Weakly basic drugs are known to become supersaturated upon transfer from an acidic to a neutral environment [6]. Supersaturation was observed in both fasted and PPI state in all HVs, as depicted in Fig. 4. Overall, the DS in the fasted state, although more variable, was higher [Mean DS_{max} = 6.52, SD ± 5.97 (n = 5)] as the shift in pH (and thus solubility) from stomach to duodenum was more pronounced. Lower maximal degrees of supersaturation [Mean DS_{max} = 3.13, SD ± 1.25 (n = 5)] were observed with concomitant PPI use owing to a less drastic pH shift. Supersaturation is an unstable state and precipitation is inevitable [5]. The precipitation kinetics of indinavir can differ in the fasted state versus the PPI state due to the presence of different amounts of solid drug particles.

The intake of a PPI resulted in a substantial reduction in intraluminal indinavir concentrations compared to the fasted state. This reduction is most pronounced in the stomach (black lines in Fig 1a versus Fig 1b) and also present in the duodenum (black lines in Fig 1c vs d). The differences between the fasted and the PPI condition are obvious when comparing the AUC_{0-3h} of the respective gastric and duodenal concentration-time profiles (Fig. 2b and d). As mentioned in Section 1, there is a known interaction between PPI and indinavir causing a reduced bioavailability [18,19]. Vice versa, *in vivo* studies in beagle dogs (low basal gastric secretion) have shown an increase in the bio-availability of the indinavir base when co-administrated with a citric acid solution (pH 2.5) [9]. As the small intestine is the main site of absorption for drugs, lower duodenal drug concentrations result in a reduced driving force for absorption [22,26]. Therefore, it is likely that the observed PPI-induced reduction in duodenal indinavir concentrations is the source of the reported reduction in systemic exposure. Our data further indicate that the reduced duodenal concentrations cannot be solely attributed to the intraduodenal pH, which is comparable with and without PPI intake (inserts in Fig. 1c and d). It seems that the elevated gastric pH causes reduced dissolution and/or increased precipitation of indinavir in the stomach, resulting in reduced intraduodenal concentrations. Thus, the stomach has a crucial role in determining the bio-availability of indinavir. Walravens et al. previously observed a good correlation between systemic and gastric concentrations of posaconazole, likewise indicating that gastric dissolution of a poorly soluble weak base dictates intestinal absorption [27].

3.2. Fasted state versus fed state

Mean gastrointestinal concentration-time profiles of indinavir following administration of Crixivan® in the fed state are depicted in Fig. 5. Compared to the fasted state (with or without PPI), variability in intraluminal concentrations appeared to be lower in postprandial conditions. Presumably, the large volume (400 mL) of Ensure® Plus created a more similar intraluminal fluid volume and composition among HVs. In all volunteers, the consumption of Ensure® Plus resulted in an immediate rise in gastric pH. Over the course of three hours, the pH gradually decreased back to basal values (insert Fig. 5a). This is in line with observations of Van Den Abeele et al. [28]. For 3 out of 5 volunteers, the initial pH exceeded the pKa of indinavir. In all three cases, however, the stomach pH dropped below the pKa before the major part of indinavir was released from the formulation (data not shown). Gastric supersaturation in the fed state is not expected as the intragastric pH reacidifies to values below the pKa prior to substantial drug release. The average percentage of gastric indinavir in solution was similar to the fasted state (90% for the fasted state vs 83%

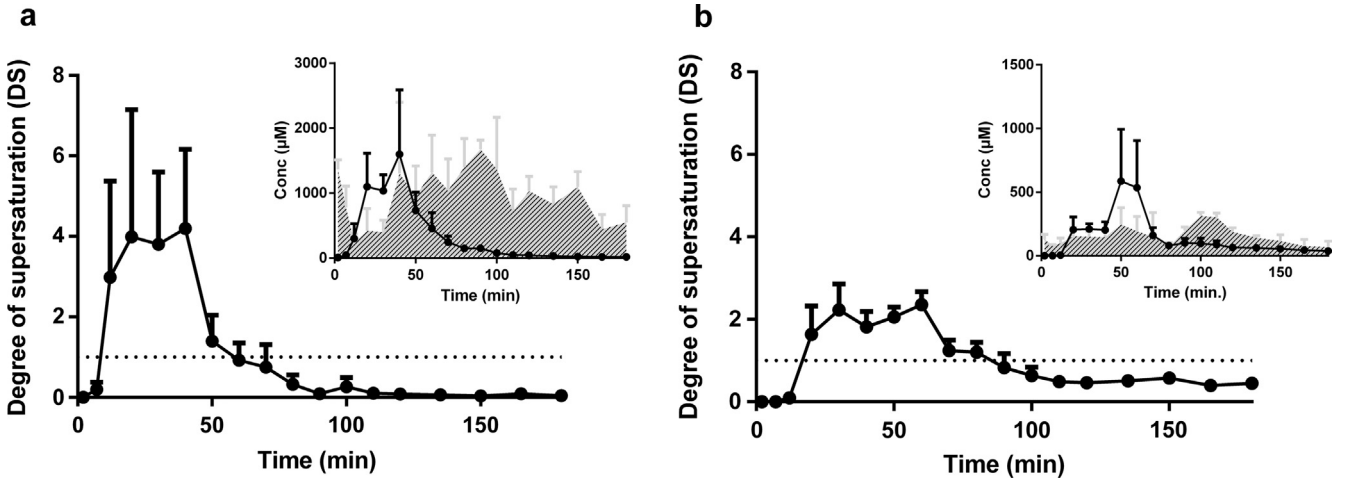

Fig. 4. Degree of indinavir supersaturation (DS) in the duodenum as a function of time after oral administration of CRIXIVAN® in (a) fasted state and (b) fasted state with concomitant use of a PPI. The DS is determined as the ratio of indinavir concentration to the indinavir solubility in each individual fluid sample. The dashed line (DS = 1) represents a saturated concentration. The inserts depict the duodenal indinavir concentration (black lines) and solubility (banded area) as a function of time (mean ± S.E.M., n = 5).

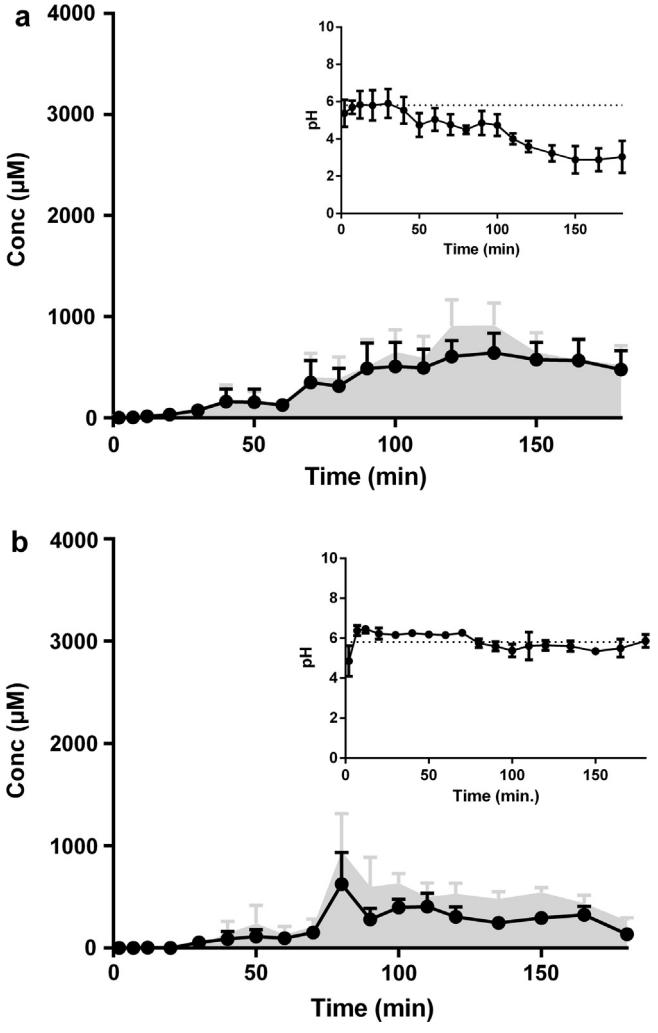

Fig. 5. Gastric (a) and duodenal (b) concentration-time profiles for indinavir following administration of Crixivan® in fed state. Black lines and gray areas represent the dissolved and total indinavir content (solid + solute expressed as concentration), respectively. Inserts depict the corresponding pH profiles as a function of time. The dashed line represents the pKa of indinavir (5.8) (mean ± S.E.M., n = 5).

for the fed state). Based on these observations it seems highly unlikely that an increased gastric indinavir precipitation due to a higher gastric pH causes a reduced systemic exposure as Yeh et al. suggested [22]. Notable is the delayed and more gradual drug release in the fed state. Lower indinavir concentrations are observed for a longer period compared to the fasted and PPI state. The average gastric T_{max} in the fed state is 128 min (SD ± 25 min) versus 34 min (SD ± 15 min) in the fasted state. A food induced delay in tablet disintegration has been observed with other formulations owing to changes in intraluminal viscosity, precipitation of food ingredients on the tablet, reduced water diffusivity and/or local changes in hydrodynamics [29–31]. Similar mechanisms might apply to the Crixivan® capsule. Indinavir concentrations could still be observed in both stomach and duodenum up to 3 h. This can be attributed to the slower emptying of gastric contents in the fed state [32]. Consequently, the average duodenal T_{max} was increased from 32 min (SD ± 18 min) in the fasted state to 105 min (SD ± 35 min) in the fed state. In agreement with the observed increase in gastric and duodenal T_{max}, Yeh et al. reported a 3-fold increase in systemic T_{max} when indinavir was given with a solid high-fat test meal compared to fasted state [22]. The average percentage of intraduodenal indinavir dissolved was similar in fasted and fed state (69% versus 61%, respectively). The intraduodenal DS as a function of time in the fed state is depicted in Fig. 6. Limited supersaturation is observed on later time points in accordance with the observed gastric and duodenal lag time. The maximal DS observed is similar to the PPI condition as the pH shift from stomach to duodenum will also be less drastic compared to the fasted state. Though indinavir was still present after 3 h, the average AUC_{0-3h} of the duodenal concentration-time profile in the fed state already reached 69% (SD ± 25%, n = 5) of the fasted state AUC_{0-3h}. Consequently, it seems unlikely that reports on reduced systemic exposure in the fed state can be explained by reduced duodenal concentrations [20–22]. The ingestion of a liquid meal will induce temporary changes in the composition of the intestinal fluids. Concentrations of bile salts, phospholipids, lipids and lipid degradation products will all increase [33]. These changes will be even more pronounced in a typical bioequivalence study where an FDA standard meal is consumed (larger volume and higher caloric load compared to 400 mL of Ensure® Plus) [34]. This intraluminal increase in solubility-enhancing compounds can hamper indinavir absorption by contributing to the micellar

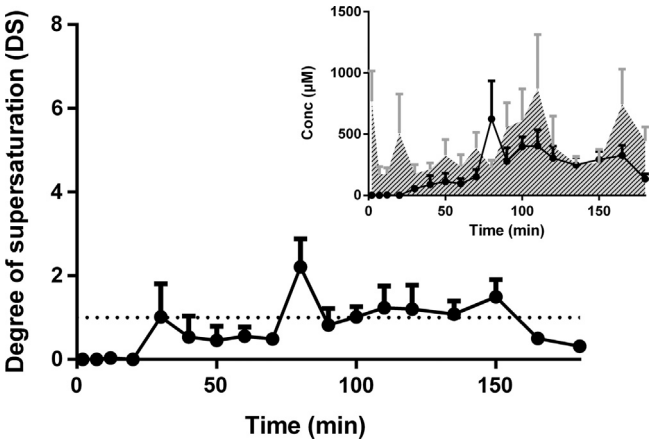

Fig. 6. Degree of indinavir supersaturation (DS) in the duodenum as a function of time after oral administration of CRIXIVAN® in the fed state. The DS is determined as the ratio of indinavir concentration to the indinavir solubility in each individual fluid sample. The dashed line (DS = 1) represents a saturated concentration. The insert depicts the duodenal indinavir concentration (black lines) and solubility (banded area) as a function of time (mean ± S.E.M., n = 5).

entrapment of the drug [35]. Holmstock et al. already observed that only a small fraction of dissolved indinavir is available for absorption from spiked fed state human intestinal fluids (FeHIF) [11]. This was confirmed using relevant aspirated intestinal samples from one HV who had taken Crixivan®. The bioaccessible fraction of indinavir was determined from one fasted and one fed state aspirate of HV 04 with similar concentrations (711 µM and 689 µM, respectively). The result is depicted in Fig. 7. Despite similar indinavir concentrations in solution, the bioaccessible fraction of indinavir appeared 2.6-fold lower in the fed state sample compared to the fasted state sample (based on the permeated fraction after 2 h). This decrease in apparent permeability of a drug with increasing the presence of colloidal species has been observed extensively for BCS class II drugs [36,37]. This difference in bioaccessible fraction of indinavir may cause a reduced systemic exposure irrespective of similar duodenal concentrations.

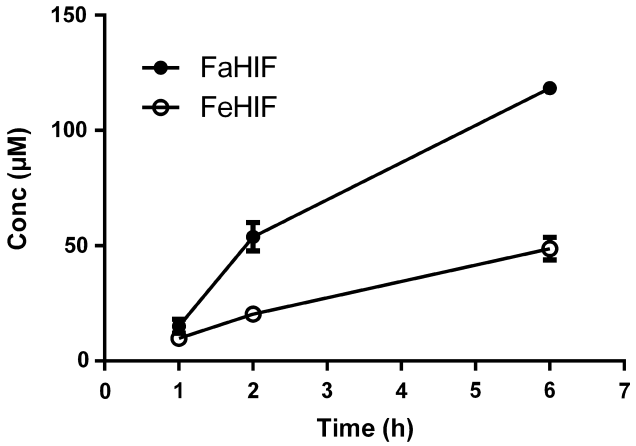

Fig. 7. Indinavir permeation from a fasted and fed state sample across an artificial membrane as a function of time. The acceptor compartment consisted of a phosphate buffer with pH and osmolality equal to the donor samples. Fasted and fed donor samples originated from the same HV and had similar indinavir concentrations (fasted state: 711 µM, fed state: 689 µM). Values are mean ± SD (n = 3).

4. Conclusion

The intraluminal dissolution, supersaturation and precipitation behavior of the weakly basic drug indinavir were investigated. The fast dissolution of the sulfate salt and excellent solubility of the base resulted in a nearly complete dissolution of the drug in a fasted stomach. Concomitant PPI use resulted in altered drug release from the capsule and a smaller fraction of total indinavir being dissolved. In fed state conditions, indinavir release appeared delayed and more gradually, though with a similar fraction of indinavir dissolved as in the fasted state. Gastric supersaturation was only relevant in a neutral stomach environment. Regardless of dosing conditions, less indinavir was dissolved in the duodenum compared to the stomach. Duodenal supersaturation was observed in all three testing conditions. The highest maximal DSs were observed in the fasted state. Overall, new insights were gained into the mechanisms underlying earlier reported interactions with concomitant PPI use and food intake. This study clarifies the role of the stomach in the interaction between PPI and indinavir. The acidity of the stomach plays a crucial role in determining the bioavailability of indinavir. Concomitant PPI administration resulted in an increased stomach pH and decreased fraction of dissolved indinavir in the stomach. In turn, this led to decreased duodenal concentrations, most likely causing reduced systemic exposure. Furthermore, this study disproves that the reported food-induced reduction in indinavir's bio-availability is caused by excessive intragastric precipitation. Reasonably high duodenal indinavir concentrations were observed which do not accord with reports on reduced systemic exposure. Most likely, the ingestion of a high-fat liquid meal causes a variety of solubility-enhancing compounds to be generated and secreted, leading to micellar entrapment and reduced absorption of indinavir.

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