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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