Pharmaceutical Research, Vol. 24, No. 10, October 2007 (© 2007)
DOI: 10.1007/s11095-007-9307-3

Research Paper

Parallel Monitoring of Plasma and Intraluminal Drug Concentrations in Man After Oral Administration of Fosamprenavir in the Fasted and Fed State

Joachim Brouwers,[1] Jan Tack,[2] and Patrick Augustijns[1,3]

Received January 26, 2007; accepted March 26, 2007; published online April 19, 2007

Purpose. The purpose of this study was to explore the feasibility of linking the pharmacokinetic profile of a drug with its gastrointestinal behavior by simultaneously monitoring plasma and intraluminal drug concentrations. Fosamprenavir, a phosphate ester prodrug of the poorly water-soluble HIV-inhibitor amprenavir, was selected as model compound.
Methods. A single tablet of fosamprenavir (Telzir®) was administered to 5 volunteers in the fasted and fed state (simulated by intake of a nutritional drink). Gastric and duodenal fluids were aspirated in function of time and characterized with respect to the concentration of (fos)amprenavir, inorganic phosphate and pH. In parallel, blood samples were collected and analyzed for amprenavir.
Results. The observed plasma concentration-time profiles suggested a food-induced delay in the absorption of amprenavir: in the fed state, mean t_{max} increased by more than 150 min compared to the fasted state. A similar delay was seen in the duodenal appearance of fosamprenavir (concentrations in mM-range) and, after dephosphorylation, amprenavir (concentrations below 160 μM). This observation could be related to the behavior of fosamprenavir in the stomach. In the fasted state, gastric dissolution of fosamprenavir started immediately, resulting in a C_{max} of 4 ± 2 mM after 43 ± 15 min; however, in the fed state, the fosamprenavir concentration remained below 20 μM for the first 90 min after drug intake. The postponed gastric dissolution may be attributed to a food-induced delay in tablet disintegration.
Conclusion. For the first time, the pharmacokinetic profile of a drug was monitored in parallel with its gastrointestinal concentrations. The observed food effect in the plasma concentration-time profile of amprenavir after intake of its phosphate ester prodrug could be related to a food-induced delay in gastric dissolution of fosamprenavir.

KEY WORDS: (fos)amprenavir; food effect; intraluminal drug behavior; pharmacokinetics; tablet disintegration.

INTRODUCTION

The absorptive phase of the plasma concentration-time profile after oral drug administration is determined by drug and formulation behavior inside the gastrointestinal tract; in addition, hepatic first-pass extraction can further modulate drug absorption into the systemic circulation. Insight in gastrointestinal and hepatic mechanisms is therefore essential to tackle issues related to the bioavailability of orally administered drugs or drug candidates. Gastrointestinal behavior is the result of various simultaneously ongoing processes, including transit, disintegration of the dosage form, dissolution and transepithelial transport.

In order to explore the gastrointestinal behavior of drugs *in vivo*, various methods have been applied. Transit and disintegration of dosage forms can be investigated by imaging techniques including gamma-scintigraphy using radiolabeled markers (1) and magnetic marker monitoring (2). Gastrointestinal intubation with the Loc-I-Gut® catheter has been applied to study drug permeability, dissolution, metabolism and secretion in humans (3–6). Another approach which has been explored to understand gastrointestinal behavior is the aspiration and characterization of gastrointestinal fluids after oral administration of the real dosage form. This allows monitoring intraluminal conditions, including drug and excipient concentrations, that are the result of the concerted action of the above mentioned gastrointestinal processes. Using this method, intestinal theophylline concentrations in man have been determined in function of time after the intake of an immediate and a slow release formulation in man (7). Also, intestinal solubilization of the poorly water-soluble protease inhibitor amprenavir (Agenerase®) by the excipient TPGS has been reported; the results of this *in vivo* study were further integrated in *in vitro* permeability studies for amprenavir (8).

Until now, intraluminal drug concentrations were only determined in fasting conditions. The intake of food may significantly alter the gastrointestinal behavior of dosage forms as it affects various aspects of gastrointestinal physiology (e.g.

[1] Laboratory for Pharmacotechnology and Biopharmacy, Katholieke Universiteit Leuven, Gasthuisberg O&N 2–Herestraat 49, box 921 Leuven, Belgium.
[2] Center for Gastroenterologic Research, University Hospitals Leuven, Gasthuisberg–Herestraat 49, Leuven, Belgium.
[3] To whom correspondence should be addressed. (e-mail: patrick.augustijns@pharm.kuleuven.be)

transit, composition of intraluminal fluids...). Furthermore, intraluminal drug concentrations have not yet been related to plasma concentrations to explore the impact of intraluminal drug behavior on the pharmacokinetic profile. In the present study, the intraluminal behavior of the prodrug fosamprenavir (Telzir®), selected as model compound, was assessed in the fasted as well as in the fed state. In parallel, plasma drug concentrations were monitored. Fosamprenavir is a phosphate ester prodrug of amprenavir with improved aqueous solubility (9); the use of the calcium salt of this prodrug results in a reduction of the pill burden as compared to the standard formulation of amprenavir (10,11). Due to the limited transepithelial transport of fosamprenavir, intestinal absorption requires gastrointestinal dephosphorylation of the prodrug to amprenavir, mediated by intestinal alkaline phosphatase. A previous *in vitro* study showed that the conversion of prodrug to parent drug in real intestinal media leads to supersaturated solutions of amprenavir, resulting in an enhanced flux across Caco-2 monolayers (12). The conversion rate was reduced in function of decreasing pH and increasing inorganic phosphate concentration.

The purpose of the present study was to monitor the gastrointestinal behavior of fosamprenavir *in vivo* and to evaluate the influence of the fed state on this behavior. Gastric and intestinal fluids were aspirated after oral intake of the formulation in the fasted and fed state and characterized with respect to the concentrations of amprenavir and fosamprenavir, inorganic phosphate and pH. In order to evaluate whether the absorptive part of the pharmacokinetic profile can be related to the gastrointestinal behavior of the drug, plasma concentrations of amprenavir were monitored in parallel to intraluminal concentrations.

MATERIALS AND METHODS

Materials

Amprenavir (505.64 g/mol) and fosamprenavir calcium (623.64 g/mol) were kindly provided by GlaxoSmithKline (Middlesex, UK). Acetonitrile, methanol and dichloromethane were purchased from Fisher Scientific (Leicestershire, UK). BDH Laboratory Supplies (Poole, UK) provided KH_2PO_4, NaOH, HCl and NaCl. Ammonium molybdate tetrahydrate and propyl-*p*-hydroxybenzoate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Certa (Brain-l'Alleud, Belgium) supplied ascorbic acid. Water was purified with a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

Scandishake Mix® (Nutricia) was used to simulate a high-fat meal. Preparation as instructed resulted in a nutritional drink (300 ml) of 2,505 kJ, consisting of 46% fat, 46% carbohydrates and 8% proteins. The osmolarity amounted to 890 mOsm/l and the pH was 6.7.

Immediate release tablets (Telzir®), containing 700 mg of the calcium salt of fosamprenavir (corresponding to 568 mg amprenavir), were used as dosage form of fosamprenavir.

In Vitro Release of Fosamprenavir

The *in vitro* release of fosamprenavir from the Telzir®-formulation was studied in media simulating gastric conditions in the fasted and fed state. A single tablet was included in 400 ml of USP simulated gastric fluid (SGF) without pepsin (pH 1.2), phosphate buffer (KH_2PO_4 50 mM, pH 6.7) or Scandishake Mix (pH 6.7) in a USP apparatus II with rotating paddles at 100 rpm and 37°C. Samples were taken at 5, 10, 15, 20, 30, 45 and 60 min and filtered through Chromafil GF/PET-45/25 filters (consisting of a glass fibre pre-filter with pore size 1 μm and a polyester filter with pore size 0.45 μm; Machery-Nagel, Düren, Germany). A 10 μl-sample of the filtrate was diluted 100 times in a solution of KH_2PO_4 (25 mM, pH 6.5) and methanol (40:60 v/v). After centrifugation (5 min, 14,000 g), 30 μl of the supernatant was injected into the HPLC-system for analysis (see below).

In addition to the dissolution experiments, the equilibrium solubility of fosamprenavir calcium was determined in the different media described above. An excess of fosamprenavir calcium powder was added to the medium and mixed on a 3D Rocking Platform STR9 (Stuart Scientific, Staffordshire, UK) at 37°C. After 24 h, samples were centrifuged (15 min, 14,000 g, 37°C) and filtered using Chromafil GF/PET-45/25 filters, after which the filtrate was diluted and analyzed as described above.

In Vivo Studies

To study the influence of the fasted and fed state on plasma and intraluminal concentrations of (fos)amprenavir, a crossover study was performed in five healthy volunteers (two men, three women; aged between 23 and 39 years). The procedure followed the tenets of the Declaration of Helsinki and was approved by the Committee of Medical Ethics of the University Hospitals Leuven, Belgium (ML3101/ML3242). All volunteers provided written informed consent to participate in this study.

After an overnight fast (12 h), one double-lumen polyvinyl catheter [(Salem Sump Tube 14 Ch (external diameter 4.7 mm), Sherwood Medical, Petit Rechain, Belgium] was introduced via the mouth and positioned into the duodenum (D2/D3). A second catheter was positioned into the stomach. The position of both tubes was checked by means of fluoroscopy. It has previously been reported that the presence of gastric or transpyloric tubes does not influence gastric emptying or duodenogastric reflux (13).

For the experiments in the fasted state, a single tablet of Telzir® was administered with 180 ml of water. For the experiments in the fed state, 300 ml of a nutritional drink (Scandishake Mix®) simulating a high-fat meal was ingested, 20 min prior to intake of the fosamprenavir tablet. Immediately after drug intake, the volunteers were asked to walk around for 5 min, after which they were sitting in upright position in a bed during the sampling procedure. Samples of human gastric fluid and human intestinal fluid (sample volume between 1.5 and 4 ml) were aspirated every 10 min during the first hour and subsequently every 15 min for up to 4 (fasted state) or 5 h (fed state). The gastrointestinal aspirates were immediately filtered through Chromafil GF/PET-45/25 filters. The filtrate was collected on ice and the procedure to determine the concentrations of fosamprenavir and amprenavir was initiated (see below). After measuring the pH (Hamilton Slimtrode, Bonaduz, Switzerland), the filtrate was stored at −30°C. Neither amprenavir nor fosamprenavir did adsorb to the catheters or the filter material used (data not shown).

The amount of drug (fosamprenavir and amprenavir) sampled from the gastrointestinal tract was estimated based on the volume and the measured drug concentrations per sample. Cumulated amounts from all samples varied between 6.3 and 9.3% of the administered dose in the fasted state and between 1.2 and 5.8% in the fed state.

In parallel to the sampling of gastrointestinal fluids, venous blood samples were collected in heparinized tubes (BD Vacutainer systems, Plymouth, UK) at 0, 20, 40, 60, 80, 100, 120, 150, 180, 210, 240, 300, 360 and 420 min after drug intake. Blood samples were stored at −30°C until analysis.

Analysis of Fosamprenavir and Amprenavir in Gastrointestinal Fluids

After aspiration and filtration of the gastrointestinal fluid samples as described above, 10 μl of the filtrate was immediately diluted 100 times in an ice-cold solution of KH_2PO_4 (25 mM, pH 6.5) and methanol (40:60 v/v), thereby arresting the possible conversion of fosamprenavir to amprenavir. After centrifugation of the diluted solution (5 min at 14,000 g), fosamprenavir and amprenavir concentrations in the supernatant were measured by reversed-phase HPLC and fluorescence detection. A volume of 50 μl was injected into a Waters HPLC system consisting of a 600E controller and pump, a 717plus autosampler and a Novapak C-18 column (Waters, Milford, MA). Fluorescence signals (excitation 268 nm, emission 347 nm, gain 10) were detected on a Jasco FP-1520 fluorescence detector (Tokyo, Japan). The column was equilibrated with a mobile phase consisting of 25 mM KH_2PO_4-buffer (pH 6.5) and methanol (70:30 v/v). After injection, the concentration of methanol was increased up to 60% over 1 min. Retention times of fosamprenavir and amprenavir amounted to 7.9 and 11.5 min, respectively. Between elution of fosamprenavir and amprenavir, the gain of the fluorescence detector was increased from 10 to 100. After elution, the column was flushed with acetonitrile:water (80:20 v/v) during 3 min and re-equilibrated with mobile phase during 4 min. The flow was maintained at a rate of 1.3 ml/min.

Calibration curves were made using a solution of KH_2PO_4-buffer and methanol (40:60) supplemented with blank intestinal fluid 1/100 and spiked with fosamprenavir and amprenavir. They were linear between 0.08 and 30 μM for fosamprenavir and between 0.008 and 3 μM for amprenavir. Samples were diluted to fit in this range. Precision and accuracy were assessed by analyzing standard samples ($n = 5$) at 0.08, 0.8, 8 and 30 μM for fosamprenavir and at 0.008, 0.08, 0.8 and 3 μM for amprenavir. The determination of the intraday repeatability resulted in relative standard deviations below 5.6% at all concentrations, both for fosamprenavir and amprenavir. The relative error remained below ±2.5% for fosamprenavir (except for −9.2% at 0.08 μM) and below ±5.0% for amprenavir. The intraday repeatability of the whole analytical procedure was also assessed by repeated analysis ($n = 3$) of selected aspirated gastric and intestinal samples, resulting in relative standard deviations below 5% for both amprenavir and fosamprenavir.

Analysis of Amprenavir in Blood Samples

Before HPLC/fluorescence analysis, amprenavir was extracted from the venous blood samples. After dilution of 300 μl blood sample with 450 μl KH_2PO_4 (0.1 M pH 6.0), 60 μl internal standard solution (propyl-*p*-hydroxybenzoate 10 μg/ml) was added. After extraction with 8 ml dichloromethane and centrifugation (4,000 g, 10 min), the water layer was discarded and the organic layer evaporated to dryness under a gentle stream of air. The residue was dissolved in 200 μl of a solution of KH_2PO_4 (25 mM, pH 6.5) and methanol (40:60 v/v). The suspension was centrifuged (14,000 g, 5 min) and the supernatant was injected into the HPLC system and analyzed in the same way as described above.

To determine concentrations, a calibration curve was generated with spiked blank human blood and treated in the same way as the samples. Linearity was obtained between 0.06 and 12 μM. Interday precision and accuracy were assessed with 5 replicates of standard samples at 0.06, 1 and 12 μM. The mean relative error amounted to −6.9, −1.0 and +1.7% at 0.06, 1 and 12 μM, respectively. Determination of the interday repeatability resulted in relative standard deviations below 9% over the linear concentration range.

Analysis of Inorganic Phosphate in Intestinal Fluid

The determination of inorganic phosphate concentrations in human intestinal fluids was based on the formation of a blue phosphate–molybdate complex. Intestinal fluid sample (100 μl) was diluted in 900 μl HCl 1 M and centrifuged for 5 min at 14,000 g. After addition of 150 μl of the supernatant to 1 ml of HCl 1 M, the complex-forming reaction was initiated by adding 150 μl ammoniummolybdate solution (2.5% in water) and 150 μl ascorbic acid solution (4% in water). After 90 min at room temperature, the absorbance was measured at 820 nm. The calibration curve, made with KH_2PO_4 in water, was linear over the concentration range of 0.2–10 mM. The assessment of intraday repeatability resulted in a relative standard deviation ($n = 5$) of 5.3% at 0.3 mM and 1.1% at 9.0 mM. Deviations from the theoretical concentration amounted to +7.5% at 0.3 mM and +1.8% at 9.0 mM.

Data Presentation and Statistical Analysis

All concentration-time profiles are presented as mean ± sd for five subjects, except for duodenal profiles in the fed state ($n = 4$). The duodenal profile of one volunteer in the fed state was omitted due to movement of the catheter back into the stomach (as observed by fluoroscopy). The parameters t_{max}, C_{max} and AUC of the concentration-time profiles in the fasted and fed state were compared using paired t-tests. Differences were considered statistically significant at $p < 0.05$. As a full pharmacokinetic analysis was beyond the scope of this study, the study design did not allow the calculation of further pharmacokinetic parameters (limited sampling time).

RESULTS AND DISCUSSION

Plasma Concentration-time Profiles

Figure 1 illustrates the mean plasma concentrations of amprenavir in function of time after oral intake of an immediate release tablet containing fosamprenavir calcium (700 mg) in fasting and fed conditions. Fosamprenavir could

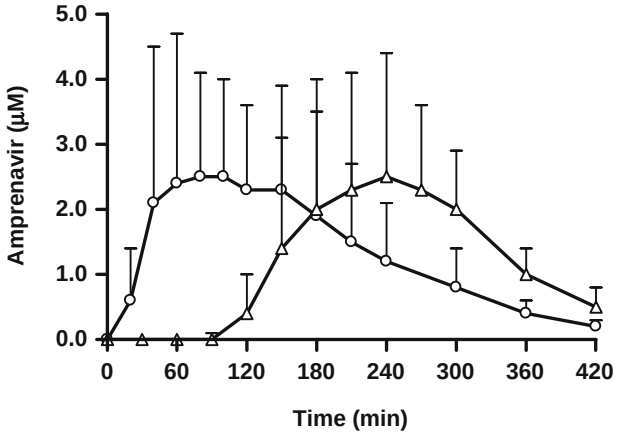

Fig. 1. Plasma concentration-time profiles for amprenavir in the fasted (*open circle*) and fed (*open triangle*) state after oral administration of an immediate release tablet containing 700 mg fosamprenavir. Results are expressed as mean ± sd, $n = 5$.

not be determined in plasma: it has been reported that the gastrointestinal tract is the primary site for fosamprenavir hydrolysis and that the systemic exposure to intact prodrug is less than 3% of the exposure to amprenavir (9). In the fasted state, absorption appeared to be relatively fast: maximum plasma concentrations (between 1.1 and 6.4 μM) were obtained after 82 min (range 40–150 min). After the intake of the nutritional drink, no amprenavir could be detected in the systemic circulation during the first 60 min after drug intake, resulting in a significant increase in t_{max} (240 min; range 180–300). Neither C_{max} nor AUC_{0-7h} was significantly altered by the intake of food. Taking into account the administered dose in the present study (700 mg instead of a normal dose of 1,400 mg), these plasma concentration-time profiles are in agreement with previously reported pharmacokinetic studies (10,11). However, the delay in amprenavir absorption after intake of a standard high-fat meal was less pronounced (up to 105 min, (10)) compared to the present study.

Gastrointestinal Concentration-time Profiles

In parallel to blood sampling, gastrointestinal samples were aspirated after intake of fosamprenavir in the fasted or fed state. Figures 2 and 3 show gastric and duodenal concentration-time profiles of fosamprenavir and amprenavir, respectively. Table I reports the t_{max}, C_{max} and AUC-values of these profiles

Fosamprenavir

In the fasted state, gastric dissolution of fosamprenavir started immediately (consistent with a conventional immediate release formulation) and resulted in maximum fosamprenavir concentrations (ranging from 2.4 to 6.2 mM) within one hour after drug intake (Fig 2a). After 10 min, fosamprenavir was observed in the duodenum (Fig. 2b), where maximum concentrations were slightly lower (up to three times) as compared to these observed in the stomach. This can be attributed to dilution by bile and pancreatic secretions and intestinal conversion of fosamprenavir to its parent drug (see below).

In the fed state, the gastric fosamprenavir concentration remained below 20 μM for 60–90 min after drug intake, which was accompanied with a significant increase in t_{max} (ranging from 90 to 160 min). The observed lag-time clearly indicates a food-induced delay in gastric dissolution. A similar delay was observed in the duodenal appearance of fosamprenavir. This intraluminal behavior may contribute to the postponed amprenavir absorption in the fed state, as observed in the plasma concentration-time profile (Fig. 1).

In three out of five volunteers, gastric, but not duodenal, fosamprenavir concentrations were significantly lower in the fed state compared to the fasted state. This suggests that dissolution of fosamprenavir was incomplete before gastric emptying and continued in the duodenum. The comparable duodenal fosamprenavir concentrations in fasted and fed conditions may contribute to the similar extent of amprenavir absorption in these two conditions (Fig. 1).

Amprenavir

Compared to intraluminal fosamprenavir concentrations (mM-range), the observed amprenavir concentrations were much lower (μM-range, Fig. 3). In the fasted as well as in the

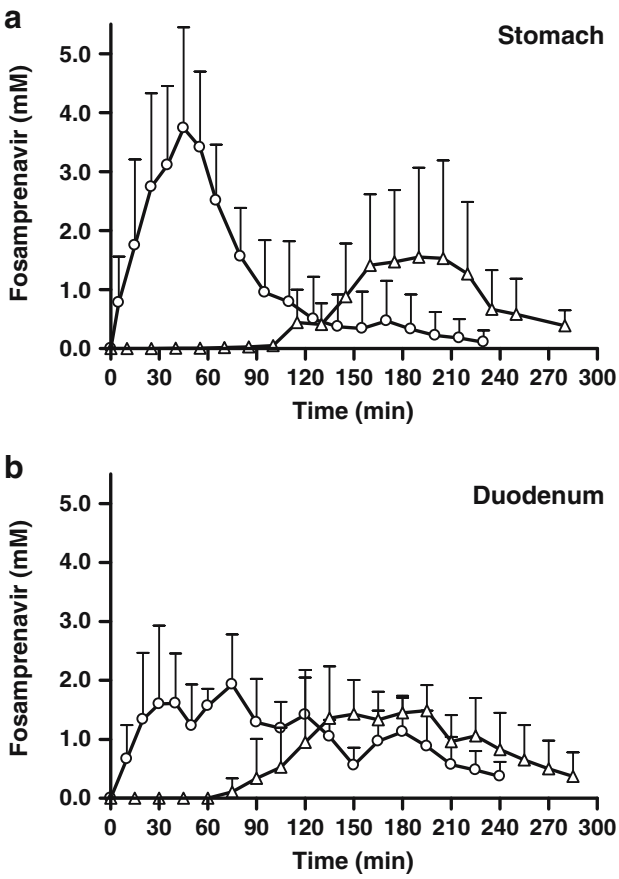

Fig. 2. Gastrointestinal concentration-time profiles for fosamprenavir in the fasted (*open circle*) and fed (*open triangle*) state after oral administration of an immediate release tablet containing 700 mg fosamprenavir: **a** stomach, **b** duodenum. Results are expressed as mean ± sd ($n = 4$ or 5).

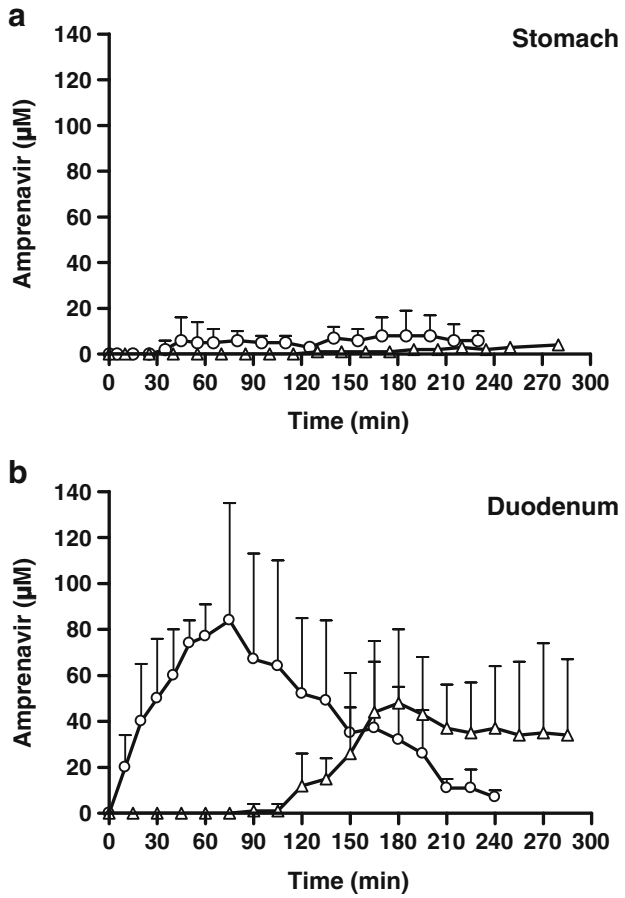

Fig. 3. Gastrointestinal concentration-time profiles for amprenavir in the fasted (*open circle*) and fed (*open triangle*) state after oral administration of an immediate release tablet containing 700 mg fosamprenavir: **a** stomach, **b** duodenum. Results are expressed as mean ± sd ($n = 4$ or 5).

fed state, the gastric concentration of amprenavir remained below 10 μM (Fig. 3a); only in one subject a concentration up to 30 μM was reached, which might be attributed to limited reflux of intestinal fluids. In the duodenum (Fig. 3b), the amprenavir concentration was higher (up to 160 μM in the fasted state), corresponding to conversion of fosamprenavir to its parent drug by intestinal alkaline phosphatase (9). In the fed state, the intestinal appearance of amprenavir was delayed by more than 90 min, corresponding to the food-induced time-shift of the duodenal concentration-time profile of fosamprenavir. Despite peak-flattening, no statistically significant food-effect could be observed for the C_{max} and AUC_{0-4h} of the amprenavir concentration-time profiles.

Previously, the creation of a supersaturated amprenavir solution by dephosphorylation of fosamprenavir in aspirated human intestinal fluids was shown *in vitro* (12). The present study cannot confirm the creation of supersaturated conditions *in vivo*, as the observed intraluminal amprenavir concentration never exceeded the amprenavir solubility in human intestinal fluids [ca. 160 μM (12)]. However, the present study was not designed to precisely investigate the dephosphorylation process *in vivo*. Conversion of fosamprenavir to amprenavir at the intestinal mucosa, which is the major dephosphorylation site, may result in local high amprenavir concentrations followed by immediate transepithelial transport; hence, intraluminal amprenavir concentrations may remain moderate. It is also important to mention that, in the present study, the amprenavir concentration was only measured in the upper part of the small intestine; as phosphatase is present along the whole intestine, the amprenavir concentration might increase further down the gastrointestinal tract.

Inter-subject Variability

As depicted in the plasma concentration-time profiles (Fig. 1), considerable inter-subject variability was observed in the rate and extent of amprenavir absorption. Looking at the individual concentration-time profiles, the variability in the absorption rate can be related to the gastrointestinal behavior of fosamprenavir. Figure 4 illustrates that the time to reach the maximum plasma concentration of amprenavir is related to the time to reach the maximum duodenal concentration of fosamprenavir. This indicates that the duodenal appearance of fosamprenavir is a major rate-determining step in the absorption of amprenavir. As the duodenal appearance of fosamprenavir depends on tablet disintegration, dissolution and gastric emptying, these processes are likely to have a large impact on the rate of amprenavir absorption. In addition to a food-induced effect, variability in disintegration and dissolution may be related to differences in gastric hydrodynamics. For instance, the intragastric location of tablets has been reported to affect drug absorption (14).

Figure 4 illustrates that inter-subject variability in the rate of amprenavir absorption could partly be related to the

Table I. Parameters of the Gastrointestinal Concentration-time Profiles After Intake of the Fosamprenavir Formulation

		Fosamprenavir		Amprenavir[a]
		Stomach	Duodenum	Duodenum
t_{max} (min)	Fasted	43 ± 15	53 ± 29	66 ± 25
	Fed	178 ± 40[b]	165 ± 44[b]	214 ± 41[b]
C_{max} (mM)	Fasted	4 ± 2	2.7 ± 0.5	0.10 ± 0.04
	Fed	2 ± 1[b]	2.0 ± 0.4	0.06 ± 0.03
AUC_{0-4h} (mM·min)	Fasted	275 ± 102	255 ± 48	10 ± 5
	Fed	141 ± 118[b]	171 ± 56	4 ± 2

Results are expressed as mean ± sd ($n = 5$, except for the duodenal profiles in the fed state where $n = 4$).
[a] Due to limited amprenavir levels in the stomach, no parameters were calculated for these profiles.
[b] Significantly different ($p < 0.05$) from the corresponding result obtained in the fasted state.

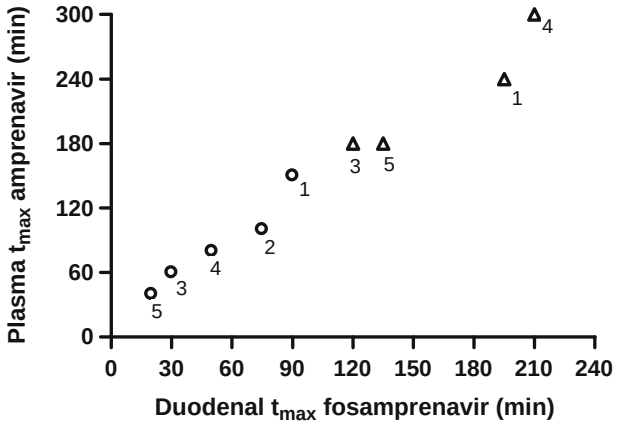

Fig. 4. Relation between plasma t_{max} for amprenavir and duodenal t_{max} for fosamprenavir in the fasted (*open circle*) and fed (*open triangle*) state. The values for t_{max} were based on the individual concentration-time profiles. The numbers indicate different subjects.

intraluminal behavior. In an attempt to further explain variability in the plasma concentration-time profiles, the relation between the extent of amprenavir absorption (plasma C_{max}, AUC) and intraluminal drug concentrations was investigated. However, no significant relation could be observed. As amprenavir is a substrate of cytochrome P450 [mainly CYP3A4 (15)], variability in the extent of absorption might be attributed to inter-individual differences in intestinal and hepatic first-pass metabolism; however, the characterization of first-pass extraction was beyond the scope of the present study.

Delayed Gastric Dissolution of Fosamprenavir in Fed Conditions

From the above mentioned results, it is obvious that the delayed absorption of amprenavir in the fed state, as reflected in the plasma concentration-time profiles (Fig. 1), corresponds to the effect of food on the appearance of fosamprenavir and amprenavir in the small intestine (Figs. 2b and 3b). The gastric concentration-time profiles (Fig. 2a) clearly indicate that this is not simply the result of delayed gastric emptying, but rather of delayed gastric dissolution of fosamprenavir in the fed state.

The delayed gastric dissolution of fosamprenavir may be attributed to altered gastric conditions in the fed state. For instance, it has been reported that the solubility of the calcium salt of fosamprenavir decreases with increasing pH (9), which implies the necessity of a rather acidic environment for dissolution of fosamprenavir calcium. This has previously been related to the slow and limited absorption of amprenavir from fosamprenavir calcium in dogs, as the gastric pH in dogs can go up to 7 in the fasted state (9). Also the decreased plasma exposure to amprenavir upon co-administration of fosamprenavir with the histamine H_2 receptor antagonist ranitidine, might be attributed to an increased gastric pH (16). In the present study, the gastric pH increased significantly (up to 6.4) after intake of the nutritional drink (pH 6.7), as can be seen in Fig. 5a. In about 2 h, the normal gastric pH was restored. To investigate the role of the gastric pH on the observed *in vivo* behavior of fosamprenavir, the dissolution of fosamprenavir from its tablet formulation was determined *in vitro* in media at pH 1.2 (USP SGF, simulating the fasted state) or pH 6.7 (phosphate buffer and nutritional drink, simulating initial conditions in the fed state). In addition, the equilibrium solubility of fosamprenavir calcium was determined in these media. At pH 1.2, dissolution was relatively fast and almost complete within 60 min (Fig. 6). In phosphate buffer at pH 6.7, a plateau level of about 60% dissolved drug was reached, consistent with the lower solubility of fosamprenavir calcium at increased pH (2.2 ± 0.1 mM in phosphate buffer versus 13.0 ± 0.1 mM in SGF). In the nutritional drink, however, dissolution of fosamprenavir was almost negligible, despite an increased solubility (5.1 ± 0.6 mM) compared to phosphate buffer.

The results of the *in vitro* dissolution tests clearly indicate that, even though dissolution of fosamprenavir is affected by the pH, the observed food-induced delay in gastric dissolution *in vivo* can not be solely attributed to the increased gastric pH in fed conditions. Tablet disintegration should also be taken into account: visual inspection at the end of the *in vitro* dissolution experiment in nutritional drink showed that the fosamprenavir tablet was largely intact. The impaired disintegration of the fosamprenavir tablet in nutritional drink was not associated with the pH (6.7) or osmolarity (890 mOsm/l) of this medium: disintegration was

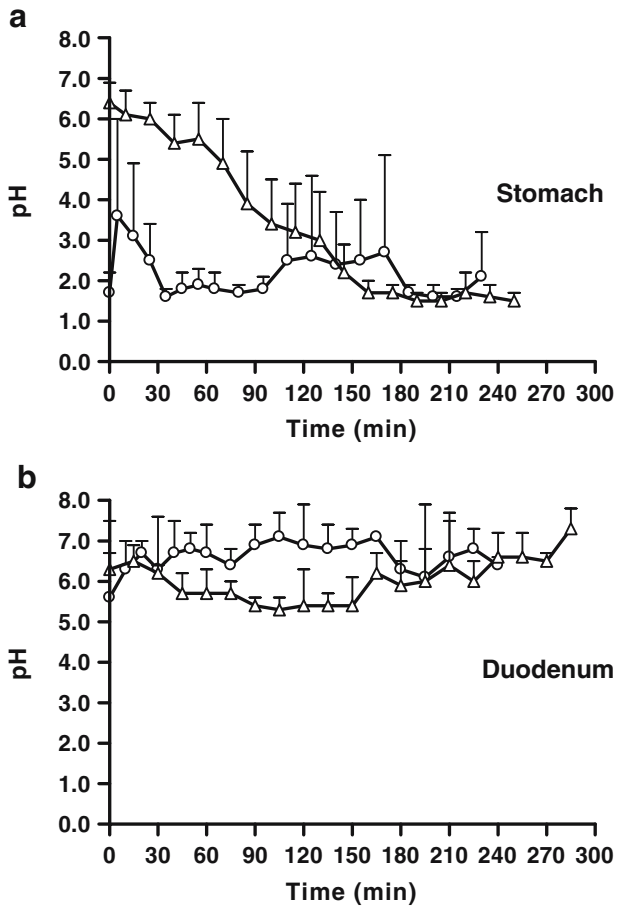

Fig. 5. Gastrointestinal time profiles of pH in the fasted (*open circle*) and fed (*open triangle*) state after administration of an immediate release tablet containing 700 mg fosamprenavir: **a** stomach, **b** duodenum. Results are expressed as mean ± sd ($n = 4$ or 5).

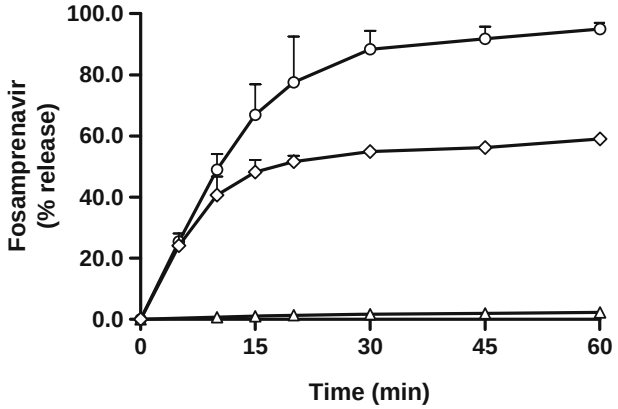

Fig. 6. *In vitro* release profiles of an immediate release tablet containing 700 mg fosamprenavir in USP SGF (pH 1.2, *open circle*), phosphate buffer (pH 6.7, *open diamond*) and nutritional drink (pH 6.7, *open triangle*). Tests were performed in 400 ml medium, at 100 rpm and 37°C. Results are expressed as mean ± sd, $n = 3$.

not affected in aqueous buffers of similar pH (Fig. 6) or osmolarity (data not shown). Abrahamsson *et al.* reported delayed disintegration of standard immediate release tablets in a nutritional drink *in vitro* and in dogs (17). This was attributed to the formation of a film of precipitated food components (mainly proteins) around the tablets, slowing down the water penetration into the tablet and preventing particles to leave the tablet. A similar phenomenon may explain the observed food-induced delay of gastric dissolution of fosamprenavir.

In contrast to well-established food effects, including non-specific increase of gastric residence time, solubilization and interactions with transport carriers and metabolizing enzymes, a food-induced delay of the disintegration of immediate release tablets is in general not considered during formulation development and evaluation, or bioequivalence assessment (e.g. granting biowaivers). However, the simultaneous assessment of gastric and plasma drug concentrations in the present study supports the potential impact of this effect on drug absorption. Delayed tablet disintegration may result in postponed drug dissolution, gastric emptying and finally drug absorption in fed conditions. These effects are unexpected for immediate release dosage forms. Therefore, it would be interesting to further explore the precise role of the tablet composition as well as the composition and form of food (e.g. nutritional drinks versus classic meals) in food effects on tablet disintegration.

Intestinal pH and Inorganic Phosphate Concentration

Intestinal dephosphorylation of a phosphate ester prodrug to its parent drug is a prerequisite for absorption. Previous *in vitro* studies in buffer medium and intestinal fluids showed that the rate of fosamprenavir dephosphorylation by intestinal alkaline phosphatase is reduced by decreasing pH and increasing inorganic phosphate concentration (12). Both variables are affected by the intake of food. Therefore, both pH and inorganic phosphate concentration of the aspirated intestinal fluid samples were determined in the present study.

The intestinal pH tended to be slightly reduced for up to 2.5 h upon intake of the nutritional drink (Fig. 5b). However, as the reduction in intestinal pH was limited, no significant effect on the dephosphorylation of fosamprenavir is expected.

The duodenal inorganic phosphate concentration was significantly increased (up to 7-fold) in fed conditions (Fig. 7), which is most likely attributed to the high level of inorganic phosphate in the nutritional drink (32 mM). A similar increase in inorganic phosphate resulted in a 2–4-fold reduction of fosamprenavir dephosphorylation *in vitro* (12). In the present study, however, the plasma and intestinal concentration-time profiles of amprenavir (Figs. 1 and 3b) did not indicate a significant reduction of amprenavir formation in the presence of high phosphate concentrations. This can be explained by the fact that maximum inorganic phosphate concentrations are reached during the first 2 h after food intake, when the amount of fosamprenavir in the intestine is limited due to delayed gastric dissolution (see above).

CONCLUSION

In the present study, the gastrointestinal behavior of fosamprenavir was investigated in the fasted and fed state by monitoring gastric and duodenal (fos)amprenavir concentrations after oral administration of fosamprenavir in man. For the first time, intraluminal concentrations were determined in parallel to plasma concentrations. This approach allowed relating the food-induced delay of amprenavir absorption, as observed in the plasma concentration-time profiles, to postponed dissolution of fosamprenavir in the fed state. The slower dissolution may be attributed to delayed tablet disintegration. The potential impact of a food-induced delay in disintegration of immediate release tablets requires further research. In addition, inter-subject variability in the rate of amprenavir absorption could be partly related to the variations in the rate of duodenal fosamprenavir appearance. Hence, the present study illustrates that the simultaneous assessment of intraluminal and plasma drug concentrations may increase insight in the impact of gastrointestinal drug behavior on absorption.

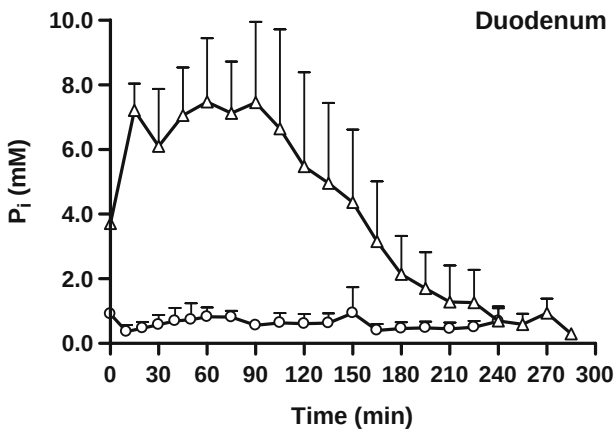

Fig. 7. Duodenal concentration-time profiles for inorganic phosphate in the fasted (*open circle*) and fed (*open triangle*) state after administration of a fosamprenavir tablet. Results are expressed as mean ± sd ($n = 4$ or 5).

ACKNOWLEDGEMENTS

This study was supported by grants from: (1) 'Fonds voor Wetenschappelijk Onderzoek', Flanders, (2) 'Onderzoeksfonds' of the K.U.Leuven, Belgium, (3) Eli Lilly and Company and (4) Organon NV. We also wish to thank Rita Vos (Center for Gastroenterologic Research, University Hospitals Leuven, Belgium) for her assistance during the *in vivo* studies.

REFERENCES

1. I. R. Wilding, A. J. Coupe, and S. S. Davis. The role of gamma-scintigraphy in oral drug delivery. *Adv. Drug Deliv. Rev*. **46**:103–124 (2001).
2. W. Weitschies, O. Kosch, H. Monnikes, and L. Trahms. Magnetic marker monitoring: An application of biomagnetic measurement instrumentation and principles for the determination of the gastrointestinal behavior of magnetically marked solid dosage forms. *Adv. Drug Deliv. Rev*. **57**:1210–1222 (2005).
3. L. Bonlokke, L. Hovgaard, H. G. Kristensen, L. Knutson, and H. Lennernas. Direct estimation of the *in vivo* dissolution of spironolactone, in two particle size ranges, using the single-pass perfusion technique (Loc-I-Gut) in humans. *Eur. J. Pharm. Sci*. **12**:239–250 (2001).
4. N. Petri, C. Tannergren, B. Holst, F. A. Mellon, Y. Bao, G. W. Plumb, J. Bacon, K. A. O'Leary, P. A. Kroon, L. Knutson, P. Forsell, T. Eriksson, H. Lennernas, and G. Williamson. Absorption/metabolism of sulforaphane and quercetin, and regulation of phase II enzymes, in human jejunum *in vivo*. *Drug Metab. Dispos*. **31**:805–813 (2003).
5. C. Tannergren, T. Knutson, L. Knutson, and H. Lennernas. The effect of ketoconazole on the *in vivo* intestinal permeability of fexofenadine using a regional perfusion technique. *Br. J. Clin. Pharmacol*. **55**:182–190 (2003).
6. E. Bergman, P. Forsell, A. Tevell, E. M. Persson, M. Hedeland, U. Bondesson, L. Knutson, and H. Lennernas. Biliary secretion of rosuvastatin and bile acids in humans during the absorption phase. *Eur. J. Pharm. Sci*. **29**:205–214 (2006).
7. J. Brouwers, F. Ingels, J. Tack, and P. Augustijns. Determination of intraluminal theophylline concentrations after oral intake of an immediate- and a slow-release dosage form. *J. Pharm. Pharmacol*. **57**:987–996 (2005).
8. J. Brouwers, J. Tack, F. Lammert, and P. Augustijns. Intraluminal drug and formulation behavior and integration in *in vitro* permeability estimation: a case study with amprenavir. *J. Pharm. Sci*. **95**:372–383 (2006).
9. E. S. Furfine, C. T. Baker, M. R. Hale, D. J. Reynolds, J. A. Salisbury, A. D. Searle, S. D. Studenberg, D. Todd, R. D. Tung, and A. Spaltenstein. Preclinical pharmacology and pharmacokinetics of GW433908, a water-soluble prodrug of the human immunodeficiency virus protease inhibitor amprenavir. *Antimicrob. Agents Chemother*. **48**:791–798 (2004).
10. C. Falcoz, J. M. Jenkins, C. Bye, T. C. Hardman, K. B. Kenney, S. Studenberg, H. Fuder, and W. T. Prince. Pharmacokinetics of GW433908, a prodrug of amprenavir, in healthy male volunteers. *J. Clin. Pharmacol*. **42**:887–898 (2002).
11. M. B. Wire, M. J. Shelton, and S. Studenberg. Fosamprenavir : clinical pharmacokinetics and drug interactions of the amprenavir prodrug. *Clin. Pharmacokinet*. **45**:137–168 (2006).
12. J. Brouwers, J. Tack, and P. Augustijns. *In vitro* behavior of a phosphate ester prodrug of amprenavir in human intestinal fluids and in the Caco-2 system: Illustration of intraluminal supersaturation. Int. J. Pharm. (2007, in press). DOI: 10.1016/j.ijpharm.2006.12.011.
13. S. A. Müller-Lissner, C. J. Fimmel, N. Will, W. Müller-Duysing, F. Heinzel, and A. L. Blum. Effect of gastric and transpyloric tubes on gastric emptying and duodenogastric reflux. *Gastroenterology* **83**:1276–1279 (1982).
14. W. Weitschies, R. Wedemeyer, O. Kosch, K. Fach, S. Nagel, E. Soderlind, L. Trahms, B. Abrahamsson, and H. Monnikes. Impact of the intragastric location of extended release tablets on food interactions. *J. Control. Release* **108**:375–385 (2005).
15. J. M. Treluyer, G. Bowers, N. Cazali, M. Sonnier, E. Rey, G. Pons, and T. Cresteil. Oxidative metabolism of amprenavir in the human liver. Effect of the CYP3A maturation. *Drug Metab. Dispos*. **31**:275–281 (2003).
16. S. L. Ford, M. B. Wire, Y. Lou, K. L. Baker, and D. S. Stein. Effect of antacids and ranitidine on the single-dose pharmacokinetics of fosamprenavir. *Antimicrob. Agents Chemother*. **49**:467–469 (2005).
17. B. Abrahamsson, T. Albery, A. Eriksson, I. Gustafsson, and M. Sjoberg. Food effects on tablet disintegration. Eur. J. Pharm. Sci. 22:165–172 (2004).