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Determination of intraluminal theophylline concentrations after oral intake of an immediate- and a slow-release dosage form

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Abstract

The purpose of this study was to evaluate a protocol which enables determining luminal drug concentrations after oral drug administration in man. Human intestinal fluids were aspirated from two sampling sites (duodenum and jejunum) at different time points after oral intake of theophylline; an immediate- and a slow-release dosage form were used to demonstrate the feasibility of discriminating between different formulations. Osmolarity and pH of the aspirates were measured and theophylline concentrations were determined by HPLC-UV. After intake of the immediate-release formulation of theophylline, duodenal maximum concentrations up to 3 mM were reached within 30 min. Theophylline appeared to be almost completely absorbed before it reached the second sampling site in the jejunum, as observed jejunal concentrations were lower than 10% of the maximal duodenal concentrations. These results are in agreement with fast dissolution and fast absorption through the intestinal mucosa, which could be expected as theophylline belongs to class I of the Biopharmaceutical Classification System. In contrast to the immediate-release formulation, administering the slow-release dosage form resulted in a gradual appearance of theophylline, reaching maximal intestinal concentrations below 300 μM. The proposed methodology can be used to assess luminal drug concentrations and to monitor the time- and site-dependent composition of intestinal fluids after intake of an oral dosage form. This approach may contribute to a better understanding of the behaviour of oral drug formulations in the gastrointestinal tract and may be exploited to further unravel the complexity of the gastrointestinal absorption process. In addition, knowledge of luminal drug concentrations may assist in the selection of drug concentrations applied in in-vitro permeability assays.

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Introduction

Absorption of orally administered drugs through the intestinal mucosa can be affected by concentration-dependent mechanisms, including metabolism and carrier-mediated absorption or efflux. During in-vitro or ex-vivo permeability estimation, the applied drug concentration is therefore of critical importance for the clinical relevance of the experiment. Presently, this test concentration is mainly based on the compound's solubility and toxicity and on analytical considerations. However, several authors point to the fact that the interpretation of the clinical impact of concentration-dependent processes during drug absorption is complicated by the ignorance of luminal drug concentrations after oral intake (Lin et al 1999; Kunta & Sinko 2004; Law et al 2004; Mouly et al 2004).

After administration of an oral dosage form, the luminal drug concentration is the result of the concerted action of various simultaneously ongoing processes. In addition to the compound's physicochemical properties and formulation factors, which determine drug release and dissolution in the gastrointestinal environment (Horter & Dressman 2001), the luminal drug concentration depends on gastrointestinal variables, including transit, hydrodynamics and gastrointestinal secretions (Scholz et al 2003). Especially for lipophilic drugs, wetting effects and solubilization by bile salts need to be considered (Pedersen et al 2000a, b; Wiedmann & Kamel 2002). Intestinal absorption and dilution by intestinal secretions, as well as propulsion and diffusion along the

gastrointestinal tract, result in a site-dependent decrease in concentration; on the other hand, active drug secretion and the enterohepatic cycle may lead to an increase in luminal drug concentration.

Several in-vivo approaches have been described to explore the fate of a drug in the gastrointestinal tract. Scintigraphic techniques permit the following of a marked compound inside the gastrointestinal tract after oral intake, thereby enabling the simultaneous study of transit, disintegration and dissolution (Digenis & Sandefer 1991; Wilding et al 2001). However, they do not allow quantification of the drug. Intubation methods have been used to study drug absorption in the gastrointestinal tract of man (Jobin et al 1985). The Loc-I-Gut perfusion method was originally proposed to investigate, in-vivo, the permeation of drugs across the intestinal barrier (Knutson et al 1989). In this method, a multi-lumen tube is used; two latex balloons are inflated to create an intestinal segment that is subsequently perfused with a drug solution. Permeability is calculated from the disappearance of drug during perfusion. This approach has become the standard method to estimate intestinal permeability in-vivo. The Loc-I-Gut method also allows assessment of intestinal drug metabolism (Petri et al 2003) and performance of in-vivo dissolution tests when the segment is perfused with a drug suspension (Bonlokke et al 1997). Observed differences between in-vitro and in-vivo dissolution of drugs indicate the impact of the complex and dynamic gastrointestinal environment (Bonlokke et al 1999, 2001).

To our knowledge, no data on intraluminal drug concentration after intake of a real oral drug formulation are presented in the literature. As stated earlier, knowledge of luminal drug concentrations would enable the selection of clinically relevant drug concentrations to be applied during in-vitro and ex-vivo permeability experiments. Furthermore, from a scientific point of view, luminal concentration–time profiles may contribute to a realistic picture of the fate of a drug in the dynamic gastrointestinal environment, including all complicating factors such as gastrointestinal transit and fluid secretions, which are accompanied by variation in osmolarity, pH and bile salt concentrations.

The study presented here was set up to develop an in-vivo protocol to sample intestinal fluid from two positions along the gastrointestinal tract after intake of an oral drug formulation. The protocol was used to monitor the luminal concentration of theophylline after oral intake of a specific formulation (i.e. an immediate- or slow-release dosage form), enabling us to explore the feasibility and the discriminative power of the methodology.

Materials and Methods

Materials

Theophylline and diprophylline were purchased from Sigma (Bornem, Belgium). Sodium taurocholate and sodium dodecyl sulfate were obtained from Fluka (Bornem, Belgium). Phospholipon 90G (lecithin) was provided by Nattermann Phospholipid Gmbh (Köln, Germany). Dichloromethane, acetonitrile and sodium acetate trihydrate were purchased from Acros Organics (Geel, Belgium), while Fisher Scientific (Leicestershire, UK) supplied us with methanol and chloroform. NaH_2PO_4, KH_2PO_4, NaOH and NaCl were provided by BDH Laboratory Supplies (Poole, UK). Isopropanol was obtained from Labscan (Dublin, Ireland) and HCl 1 M from Chem-Lab (Lichtervelde, Belgium).

The two oral dosage forms of theophylline that were evaluated in this study consisted of an immediate- and a slow-release capsule, each containing 100 mg theophylline. As slow-release formulation, Xanthium 200 (SMB, Brussels, Belgium) capsules were used, containing theophylline formulated as pellets (median size 1.24 mm (0.78–1.96 mm)); the dose was adjusted to 100 mg anhydrous theophylline. These pellets were coated with Eudragit NE30D that ensures the sustained release of theophylline, independent of the pH of the gastrointestinal tract. The immediate-release capsules were formulated by filling emptied Xanthium 200 capsules with 100 mg of anhydrous theophylline, European Pharmacopoeia quality (Certa, Brain-l'Alleud, Belgium).

In-vitro release of theophylline

The in-vitro dissolution profiles of capsules containing 100 mg theophylline (immediate- or slow-release formulation) were obtained using the USP apparatus II at 37°C, with a paddle rotating at 50 rev min^{-1}, in 500 mL of standard dissolution media. Capsules were attached in a sinker. Samples were taken at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min and analysed by HPLC/UV. To simulate gastric fluid, HCl 0.01 M and simulated gastric fluid (SGF, consisting of 0.01 M HCl, 34.2 mM sodium chloride and 8.7 mM sodium dodecyl sulfate) were used as dissolution media. Intestinal fluid was simulated with phosphate buffer (KH_2PO_4 50 mM, pH 6.5) or fasted state simulated intestinal fluid (FaSSIF, containing 3 mM sodium taurocholate and 0.75 mM lecithin in phosphate buffer (NaH_2PO_4 28.5 mM, NaCl 100 mM, pH 6.5)). The pH of both dissolution media simulating intestinal fluid was adjusted to 6.5, corresponding to the pH of FaSSIF, suggested as biorelevant medium to better simulate the in-vivo dissolution process in the upper small intestine (Galia et al 1998). It is obvious that small changes in the selected pH may have a high impact on the dissolution profile, especially if pH-dependent swelling or dissolution of the formulation coating controls drug release. This pH dependency may add to in-vivo variability and may compromise in-vitro/in-vivo correlations.

In-vivo studies with theophylline dosage forms

The in-vivo study followed the tenets of the Declaration of Helsinki and was approved by the Committee of Medical Ethics of the University Hospitals Leuven, Belgium. All subjects provided written informed consent to participate in the study.

After an overnight fast, two double-lumen polyvinyl catheters were introduced via the mouth and positioned

into the gastrointestinal tract. One catheter (Salem Sump Tube 14 Ch (external diameter 4.7 mm); Sherwood Medical, Petit Rechain, Belgium) was located in the duodenum, the other catheter (Bowel Decompression Catheter 16 Ch (external diameter 5.3 mm); Rusch, Lurgan, UK) 90 cm more distally in the jejunum. It was the standard procedure to check the position of both tubes before and after the experiment by means of fluoroscopy. These double-lumen tubes allowed sampling of luminal fluid (by means of a syringe), without creating underpressure in the gastrointestinal tract. After a stabilization period of 30 min, during which blank intestinal fluid was collected from the two catheters, subjects were administered a capsule containing 100 mg of theophylline (immediate- or slow-release formulation) with 180 mL of water. Samples of intestinal fluid were collected on ice from the two positions at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225 and 240 min after drug intake. They were immediately centrifuged at 4000 rev min^{-1} for 20 min (4°C) and kept on ice before theophylline analysis on the same day. The pH and osmolarity (Model 3D3 osmometer; The Advanced Instruments, MA) of each sample was measured.

To compare the two dosage forms of theophylline, this procedure was performed with one formulation and repeated at least one week later with the other formulation in 4 caucasian subjects (3 men and 1 woman, aged 23–42 years). To estimate the intra-individual variability between luminal concentration–time profiles, the procedure was performed three times with the immediate-release formulation in a fifth male subject (aged 35 years).

Theophylline extraction from intestinal fluid samples

Before HPLC/UV analysis, a liquid–liquid extraction was performed on the intestinal fluid samples. A 50-μL sample was diluted in 450 μL KH_2PO_4 0.1 M, pH 6.0, after which 100 μL internal standard solution (diprophylline 50 μM) was added. After extraction with 2 mL chloroform–isopropyl alcohol 1:1 (30 s vortex) and centrifugation (4000 rev min^{-1}, 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 mobile phase, of which 100 μL was injected into the HPLC system.

Theophylline analysis

Theophylline concentration was measured by reversed-phase HPLC and spectrophotometric detection. The HPLC system consisted of a model 600E controller and pump, a model 717plus autosampler and a model 2487 dual λ absorbance detector (Waters, Brussels, Belgium). The column used was a Waters Novapak C-18 column (4 μm) and the mobile phase was 10 mM KH_2PO_4–acetonitrile–methanol (900:25:90 v/v). The flow was maintained at 2 mL min^{-1}. Absorbance at 271 nm was monitored and the observed peaks were integrated using a personal computer running Waters Millennium 32 Chromatography software. Retention times of theophylline and the internal standard were 9.5 and 10.6 min, respectively. After elution of both compounds, the column was flushed with acetonitrile–water (80:20 v/v) for 2 min and re-equilibrated with mobile phase for 4 min.

Concentrations were determined by comparison with a calibration curve using blank intestinal fluid, spiked with theophylline from a stock solution in dimethyl sulfoxide (DMSO) and treated in the same way as described above. For intestinal fluid, calibration curves were linear over the concentration range of 1 μM (quantification limit) to 1 mM. Samples with higher concentrations were first diluted to fit in this range. Intraday repeatability of the extraction and analysis procedure, respectively determined at 8 and 800 μM, resulted in relative standard deviations of 1.3 and 1.4%. Deviations from the theoretical concentration amounted to 9.5 and −1.5% at 8 and 800 μM, respectively.

Statistical analysis

To evaluate the in-vitro dissolution profiles of the immediate-release formulation, a non-linear Rosin–Rammler–Sperling–Bennett–Weibull (RRSBW) regression was performed according to Langenbucher (1976). The resulting parameter t_d, corresponding to the time to reach 63.2% of dissolution, was analysed using a non-parametric Kruskal–Wallis test to compare dissolution in different media. To compare the duodenal concentration–time profiles of the two dosage forms a paired *t*-test was performed on the logarithm of the calculated $AUC_{0\text{-}4h}$ and C_{max}. In all cases $P < 0.05$ was accepted to denote significance.

Results and Discussion

In-vitro release of theophylline

In a first set of experiments, the release profiles of the immediate- and slow-release dosage forms of theophylline were determined using dissolution tests, simulating both gastric (HCl 0.01 M and SGF) and intestinal (phosphate buffer pH 6.5 and FaSSIF) conditions.

The disintegration of the capsule was determined by visual inspection: all powder or slow-release pellets were released (but not dissolved) within 10 min. Figure 1 clearly illustrates that theophylline dissolves significantly faster from the immediate-release formulation than from the slow release formulation. Less than 20% of the total dose was dissolved from the slow-release pellets after 120 min, while dissolution from the immediate-release formulation was complete after 45–90 min in the different media.

Regression analysis of the dissolution profiles of the immediate-release formulation (Langenbucher 1976) followed by a Kruskal–Wallis test on the time to reach 63.2% dissolution t_d did not reveal any statistically significant differences between the different dissolution media, indicating no significant pH or bile-salt effect (t_d (min): HCl 18 ± 3, SGF 25 ± 9, phosphate buffer 42 ± 13, FaSSIF 24 ± 11). The absence of significant effects may be

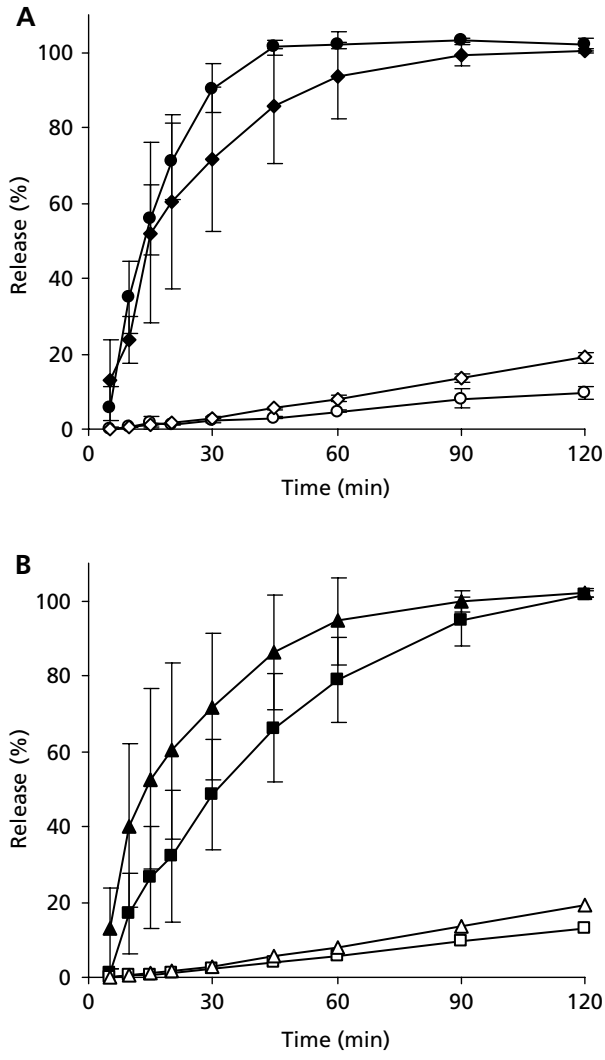

Figure 1 In-vitro release profiles of theophylline immediate-release (closed symbols) and slow-release (open symbols) formulations, each containing 100 mg theophylline. Dissolution tests were performed in 500 mL medium, at 50 rev min^{-1} and 37°C. A. Media simulating gastric fluid: HCl 0.01 M (●,○) and SGF (◆,◇). B. Media simulating intestinal fluid: phosphate buffer pH 6.5 (■,□) and FaSSIF (▲,△). Data represent average release % ± s.d., n = 3.

partly related to the high variability observed in the dissolution profiles. High variability in standard dissolution testing has been discussed by various authors (Qureshi & Shabnam 2001; Baxter et al 2005) and may be partly explained by heterogeneous and non-optimized hydrodynamic conditions.

In-vivo studies

A protocol was evaluated to measure theophylline concentrations in aspirates obtained from the gastrointestinal tract of man as a function of time after administration of an oral dosage form (immediate and slow release). Intestinal fluid was sampled from the duodenum and jejunum by two double-lumen catheters. To our knowledge, such a combined intubation technique has never been used to monitor intraluminal drug concentrations after oral drug administration.

Although some interference with normal gastrointestinal behaviour by the presence of two transpyloric tubes can not be excluded, previous studies showed negligible influence of gastrointestinal catheters on gastric emptying and secretions (Longstreth et al 1975) or on possible reflux of duodenal contents into the stomach (Go et al 1970; Rees et al 1979).

Intestinal fluid sampling

After positioning of the catheters in the gastrointestinal tract of subjects and oral administration of a capsule containing 100 mg theophylline (immediate- or slow-release formulation), intestinal fluid was aspirated at fixed time points over 4 h. The current methodology did not allow standardization of the sampled volume, which varied between 0 and 10 mL at each sampling time. The volume of fluid remaining in the catheter after sampling was re-injected in the gastrointestinal lumen.

The amount of theophylline sampled during an experiment was estimated based on the volume and the measured theophylline concentration per sample. Cumulated amounts over all samples varied between 0.6 and 1.5 mg for the slow-release formulation and between 2.1 and 8.1 mg for the immediate-release formulation, which was always less than 10% of the total dose of 100 mg.

pH and osmolarity of intestinal fluid samples

To characterize the aspirated intestinal fluid, the pH and osmolarity of all samples were measured. Mean time profiles are presented in Figure 2. The pH of duodenal samples after administration of the slow-release formulation to subject 3 appeared to be very low. As these pH values could be considered as outliers (Grubbs' test on the mean, $P < 0.01$), they were excluded from the calculation of the mean profile and were presented separately on the graph. Strong acidic pH values in the duodenum have been reported in previous studies (Woodtli & Owyang 1995; Lee et al 2004).

After administration of the capsule together with 180 mL of water, a temporary drop in osmolarity in the duodenal samples could be seen in all profiles. Mean osmolarity values amounted to 242 ± 50 mOsm in the duodenum (excluding the samples of the first hour) and 269 ± 34 mOsm in the jejunum. The pH over all time points varied between 3.5 and 9.3 (median 6.9) in the duodenum (after exclusion of the outliers) and between 3.8 and 9.4 (median 7.2) in the jejunum. These values were in agreement with previously published data on the composition of intestinal fluid in fasting subjects (Lindahl et al 1997; Pedersen et al 2000b).

Luminal concentration–time profiles for theophylline immediate-release formulation

Figure 3 shows time profiles of the theophylline concentration measured in aspirated intestinal fluid sampled at two positions (duodenum and jejunum) after intake of the

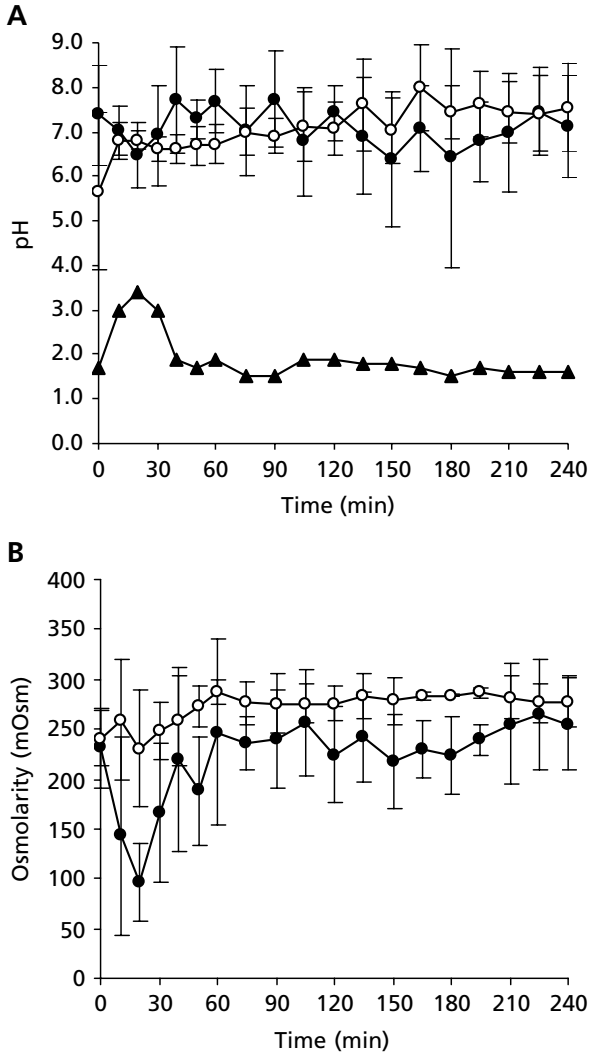

Figure 2 Time profiles of pH (A) and osmolarity (B) of samples aspirated from the duodenum (●) and jejunum (○) after oral administration of a 100-mg theophylline dosage form to healthy subjects. Data represent mean values ± s.d., n = 8, except for the duodenal pH, where n = 7. The duodenal pH profile of subject 3 after administration of the slow release formulation (▲) was considered as an outlier and excluded from the calculation of the mean.

immediate-release dosage form. In an extra set of experiments, the immediate-release formulation was administered three times to a fifth subject (concentration–time profiles are shown in Figure 4).

Administering the immediate-release formulation to subjects 1, 2 (Figure 3A, B) and 5 (Figure 4) resulted in high maximal duodenal concentrations (C_{max} 1.8–3.2 mM), observed after 20–30 min. Theoretically, complete dissolution of 100 mg theophylline in 180 mL water would result in a concentration of 3 mM. Within 90 min, duodenal concentrations decreased to less than 5% of C_{max}. These observations are consistent with almost complete dissolution of theophylline in the stomach (as suggested by the in-vitro release profiles, Figure 1A), followed by transit into the duodenum within 30 min, resulting in high peak concentrations. The time period for gastric emptying corresponds to values found in studies in which the route of non-absorbable tracers through the gastrointestinal tract is followed by scintigraphy (Argenyi et al 1995; Haruta et al 2002). At the second sampling site (90 cm more distally in the jejunum), theophylline could be detected within 10–20 min after duodenal peak concentrations, indicating relatively fast transit in the upper small intestine. Maximum jejunal concentrations (0.1–0.9 mM) never exceeded 10% of the duodenal C_{max}, except for one profile in subject 5 (up to 28%). These relatively low concentrations in the jejunum probably point to extensive absorption of theophylline before it could reach the second sampling site. In addition, dilution of the drug by diffusion in the gastrointestinal tract and by gastrointestinal secretions may contribute to lower luminal concentrations.

After intake of the immediate-release formulation by subjects 3 and 4, the concentration–time profiles had a different appearance (Figure 3C, D). In the duodenum, C_{max} was, as for the other subjects, reached after 30 min, but amounted to only 0.5 and 0.8 mM, respectively. On the other hand, concentrations remained relatively high for a longer time period. In the jejunum, no distinct maximum could be observed, and the concentration did not exceed 50 μM. Slower transit of dissolved theophylline from the stomach into the upper small intestine could be a possible explanation for this different pattern. It is conceivable that this would result in profiles similar to those observed for subjects 3 and 4, with lower but prolonged peaks in the duodenal concentration–time profile, and very low concentrations in the jejunum, as there would be more time for absorption before reaching the second sampling site due to slower transit. Striking intra- and interindividual differences in gastrointestinal transit in fasted subjects have been reported in the past (Argenyi et al 1995; Degen & Phillips 1996); however, this explanation could not be ascertained, as our current methodology enables no objective evaluation of gastrointestinal transit times.

Luminal concentration–time profiles for theophylline slow-release formulation

When we compare the concentration–time data of the immediate-release formulation of theophylline with those of the slow-release formulation (Figure 5), a completely different profile was observed. As mentioned above, slow-release pellets are rapidly released from the capsule in the stomach, but theophylline dissolution is retarded by the presence of polymethacrylate in the Xanthium slow-release pellets. Indeed, polymers of methacrylate tend to swell when placed in an aqueous medium so that the drug is only gradually released by diffusion (Wade & Weller 1994). Logically, this slow release, combined with gastrointestinal transit of the pellets, would lead to low concentrations in the duodenum and higher concentrations further down the gastrointestinal tract. This could be observed in subjects 1 and 2 (Figures 5A, B), in whom duodenal concentrations were less than 60 μM, with higher concentrations in the jejunum (up to

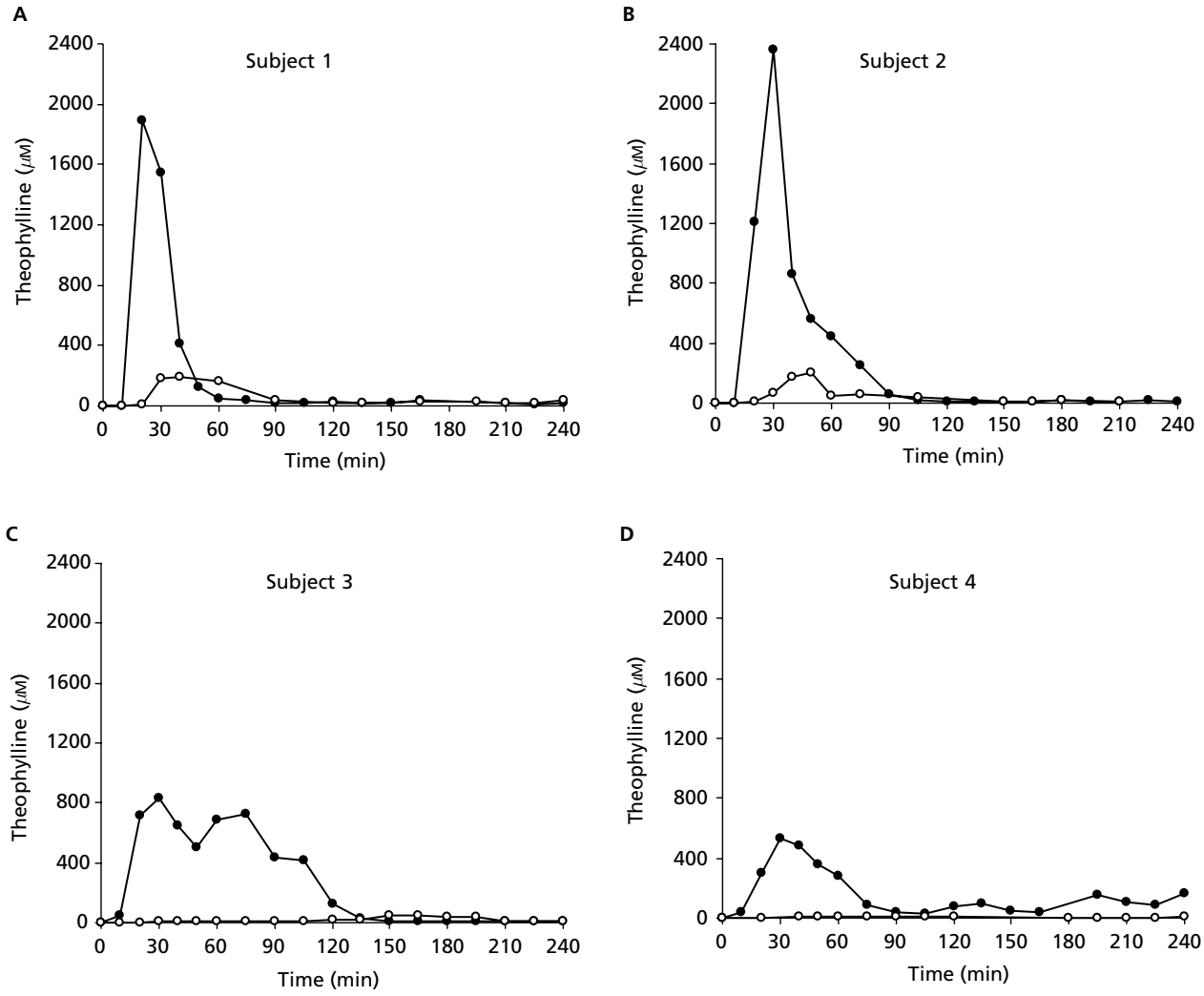

Figure 3 Time profiles of the theophylline concentration in gastrointestinal fluid sampled from the duodenum (●) or jejunum (○) of healthy subjects after oral administration of an immediate-release dosage form containing 100 mg theophylline.

300 μM). Release of theophylline from the slow-release pellets is expected to continue over a longer time period and further down the intestine, where fluid could not be aspirated in the present methodology. Literature data reveal that a slow release formulation (Xanthium) results in maximum plasma concentrations of theophylline only after 7–8 h (Rojanasthien et al 2001), in comparison with plasma peaks within the first 2 h for the immediate-release dosage form (Varshosaz et al 2000). Hence a full evaluation of the slow-release dosage form would require an extended sampling period, preferably further down the intestine.

The slow-release dosage form resulted in equally low duodenal concentrations in subject 4 (Figure 5D); however, unexpected high values were obtained in aspirates originating from the jejunum (0.8 mM at 135 min, 3 mM at 210 min). No obvious explanation can presently be given for these observations.

In subject 3, the concentration–time profile suggests a gradual release of theophylline already in the duodenum (concentrations up to 150 μM), which was accompanied by relatively low jejunal concentrations (Figure 5C). A slower gastrointestinal transit of the pellets could be at the origin of this observation; this was also suggested for the concentration–time profiles obtained after administering the immediate-release formulation to this subject.

General considerations

From the results presented so far, it is obvious that the proposed methodology can be used to construct luminal concentration–time profiles for orally administered drugs. Even though a considerable variability is observed between subjects, these profiles allow discrimination between immediate- and slow-release dosage forms of theophylline. Despite the low number of subjects, a statistically significant difference could be observed between immediate- and slow-release profiles in the duodenum for both $AUC_{0\text{-}4h}$ (5 ± 1 vs $0.9 \pm 0.8 \times 10^4$ μM min, respectively) and C_{max}

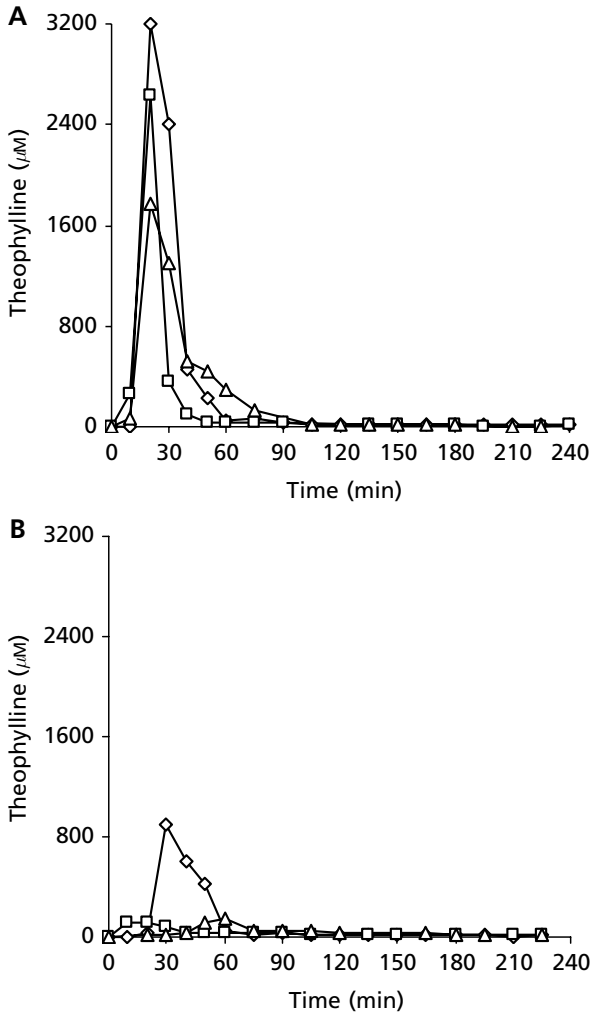

Figure 4 Intrasubject variability in luminal theophylline concentrations. The immediate-release dosage form of theophylline was administered three times (◇, □ and △) to the same subject. Concentration–time profiles were assessed in the duodenum (A) and jejunum (B).

(1.4 ± 0.9 vs 0.08 ± 0.05 mM, respectively). When the same experiment (immediate-release formulation) was repeated three times in the same subject, variability was still evident, although the profiles appeared to be similar in general (Figure 4). The observed variability should not be too surprising, taking into account the numerous parameters that affect intestinal concentrations, as described in the introduction. The current approach does not allow the evaluation of influencing factors such as the volume of intestinal fluid, gastrointestinal transit and drug absorption. On the other hand, some of the parameters that may affect drug release and dissolution in the gastrointestinal tract (e.g. the composition of intestinal fluid) can be monitored with the present methodology. In this study, we have only measured the pH and osmolarity as a function of time at the two sampling positions (Figure 2). As the release and dissolution of theophylline appeared to be relatively independent of the aqueous media (Figure 1), a significant impact of the gastrointestinal fluid composition on theophylline dissolution was not expected. Indeed, no correlation could be observed between the concentration–time data and the pH or osmolarity of the samples. The aberrant duodenal pH profile observed after administration of the slow-release formulation to subject 3 (Figure 2A) corresponds to relatively high duodenal theophylline concentrations (compared with other subjects); however, it is unlikely that a causal connection exists between these two observations, as the in-vitro studies did not reveal a faster dissolution of theophylline from the slow-release formulation at lower pH (Figure 1). For drugs with a more complex dissolution behaviour (e.g. poorly water-soluble drugs), the simultaneous measurements of pH, osmolarity and bile salt content could possibly be related to intestinal drug behaviour. For those drugs, the present methodology may bridge the knowledge generated using in-vitro dissolution tests and in-vivo bioavailability data.

It would also be interesting to combine the technique presented with concomitant blood sampling to relate intraluminal concentrations with drug appearance in the systemic circulation. However, the aim of this study was to explore the intestinal sampling procedure to improve our understanding of intraluminal formulation behaviour.

As mentioned in the introduction, a better understanding of the composition of intestinal fluids during drug absorption could be useful to increase the biorelevance of in-vitro and ex-vivo permeability experiments. More specifically, the knowledge of luminal drug concentrations after oral drug intake permits the selection of clinically relevant test concentrations to be applied during these experiments. In this study, we have demonstrated a methodology to assess these concentrations. Of course, it has to be noticed that in the gastrointestinal environment, interactions of the drug with bile salts, formulation factors or food components may occur and thus complicate the interpretation of aqueous concentrations. For instance, these interactions will lower the effective drug concentration available for transport through the intestinal epithelium. It is obvious that these complicating factors should also be considered when designing relevant permeability experiments.

Conclusion

The proposed approach allows monitoring of the time- and site-dependent composition of intestinal fluids after oral drug intake. This may contribute to a better understanding of the behaviour of oral dosage forms in the gastrointestinal tract. Moreover, the data presented in this study illustrate that the methodology permits discrimination between immediate-release and slow-release formulations, and can thus be used for the evaluation of formulation behaviour in the gastrointestinal tract. The approach may also be exploited to further unravel the complexity of the gastrointestinal absorption process. Knowledge of luminal drug concentrations can be of interest to improve the clinical relevance of in-vitro and ex-vivo permeability experiments, especially considering the impact of concentration-dependent processes on absorption.

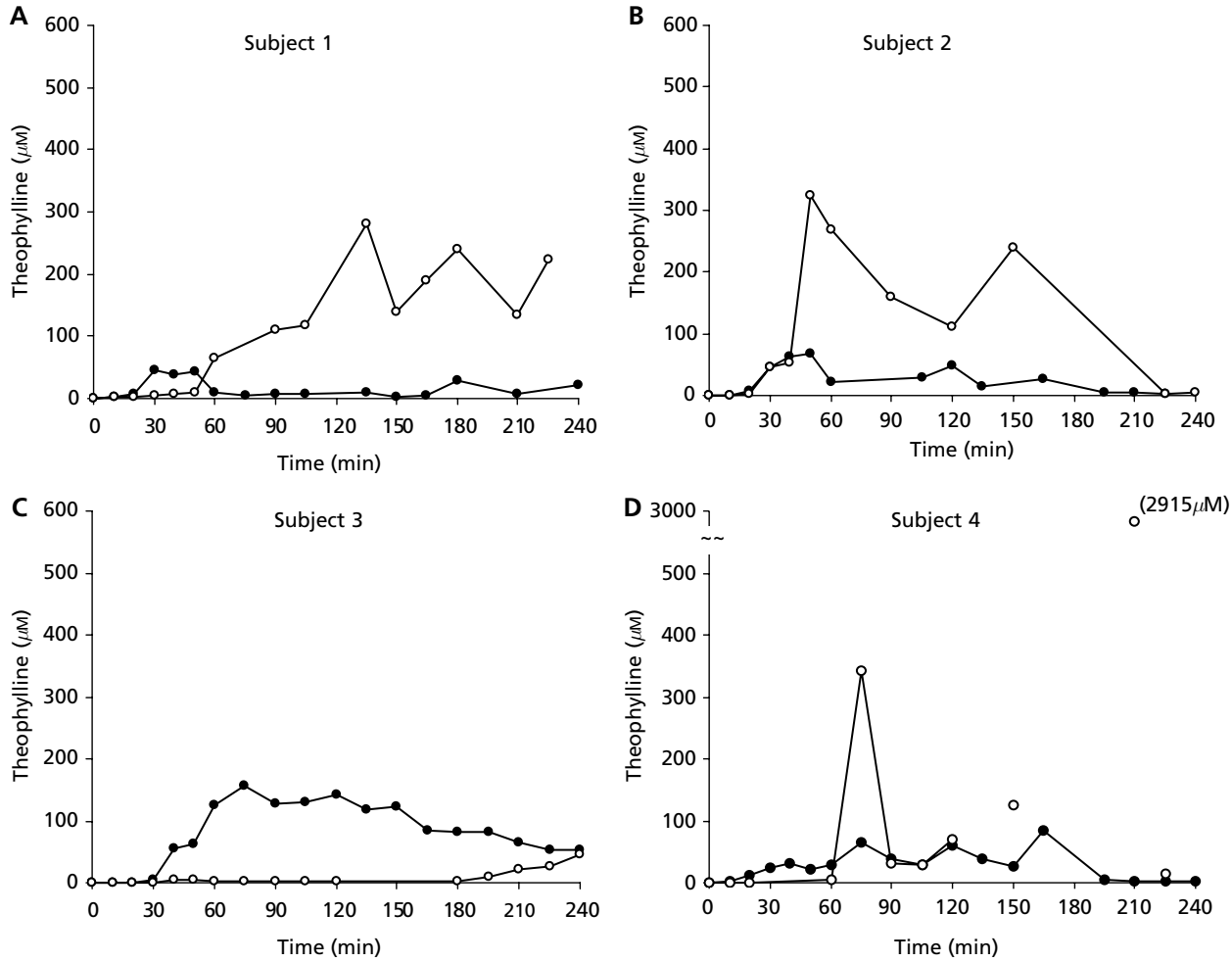

Figure 5 Time profiles of the theophylline concentration in gastrointestinal fluid sampled from the duodenum (●) or jejunum (○) after oral administration of a 100-mg slow-release dosage form to healthy subjects.

In future studies, we will attempt to further improve and strengthen the methodology by determining individual bile salts and lecithin, assessing drug plasma concentrations in parallel to luminal concentrations, extending the sampling period and increasing the number of subjects studied. Especially for monitoring intraluminal concentrations after intake of a controlled-release dosage form, we should consider positioning the second catheter even further down the gastrointestinal tract.

The results of the first experiments with two formulations of theophylline warrant further studies to assess the behaviour of low solubility compounds or to explore the effect of complicating factors, such as formulation excipients or food components.

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