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Research paper

Bioavailability of amoxicillin and clavulanic acid from extended release tablets depends on intragastric tablet deposition and gastric emptying

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ABSTRACT

The rate and extent of amoxicillin and clavulanic acid absorption from pharmacokinetically enhanced extended release (ER) tablets is strongly influenced by the intake conditions. In order to investigate the cause of the food effects, a pharmacokinetic study with simultaneous imaging of the in vivo behaviour of the ER tablets by magnetic marker monitoring (MMM) was performed. Under fasting conditions the amoxicillin AUC (1854 ± 280 μg min ml^{-1}) was significantly lower than after intake at the beginning of the breakfast (2452 ± 354 μg min ml^{-1}) or after the breakfast (2605 ± 446 μg min ml^{-1}). In contrast, clavulanic acid AUC was well comparable after tablet intake under fasting conditions and intake at the beginning of a breakfast (191 ± 46 and 189 ± 44 μg min ml^{-1}, respectively) but significantly lower following a breakfast (126 ± 71 μg min ml^{-1}). The localization data showed that the reduced bioavailability of amoxicillin under fasting conditions is due to early gastric emptying in combination with poor absorption from deeper parts of the small intestine. Prolonged gastric residence of clavulanic acid caused by intragastric tablet deposition in the proximal stomach was identified as the reason for the decreased bioavailability of clavulanic acid after tablet intake following the meal.

1. Introduction

Extended release (ER) formulations are commonly used for drugs with short elimination half-life to reduce the frequency of daily administrations and, for drugs with adverse effects related to high fluctuations in plasma concentrations, to reduce the risk of the occurrence of such effects. However, due to their long residence time in the gastrointestinal tract, drug absorption may largely be influenced by gastrointestinal transit and gastrointestinal luminal conditions. The main causes for alterations in the extent and rate of drug absorption derived from ER formulations that are related with gastrointestinal transit and gastrointestinal conditions are varying drug absorption behaviour of different parts of the intestines (absorption windows), site and motility dependent mechanical forces on the formulation and changes in the local environment such as differing pH values, buffer capacities, osmotic pressures, concentration of bile, luminal enzymes and locally available fluid volumes [1–4].

The most prominent factor influencing gastrointestinal transit as well as gastrointestinal environment is the ingestion of food. Accordingly, the susceptibility of ER formulations to alterations of gastrointestinal conditions is usually investigated in food-effect studies, where plasma concentration profiles obtained after dosage form intake under fasting and fed conditions are compared. In order to maximize the difference between fasting and fed conditions the FDA guideline on food-effect bioavailability and fed bioequivalence studies recommends to conduct food-effect studies using a high caloric and high fat test meal. The drug is administered 30 min after the start of the meal. In fasted treatments drug administration is advised to follow an overnight fast of at least 10 h [5].

An extended release (ER) tablet formulation of amoxicillin and clavulanic acid (Augmentin XR) has been introduced as a novel 'pharmacokinetically enhanced' formulation in order to increase the efficacy of therapy by maintaining plasma concentrations of amoxicillin above the MIC for an elongated period compared to conventional immediate release (IR) formulations [6]. Augmentin

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XR is a bilayer tablet, with an immediate release layer containing 562.5 mg amoxicillin and 62.5 mg clavulanic acid, and a modified release layer containing 437.5 mg amoxicillin [7]. The recommended dosage is two tablets twice daily. Amoxicillin and clavulanic acid are class III compounds of the Biopharmaceutical Classification System (BCS) with good solubility and low permeability. According to the prescribing information, it is advised that the tablets are taken at the start of a meal because absorption of amoxicillin is decreased in the fasted state and, absorption of clavanulate is decreased when the tablets are taken 30 min after a high fat meal [8]. Accordingly, ingestion of this formulation is not recommended under standard conditions of phase I clinical trials.

It was the aim of our study to determine the cause of the reported differences in the bioavailability of amoxicillin and clavulanic acid after administration of the ER tablet under fasting conditions, at the beginning of a meal and, 30 min after start of a high fat meal. For this purpose, a clinical study was performed in nine healthy volunteers where the plasma concentration profiles of amoxicillin and clavulanic acid were determined and the behaviour of the ER tablets in the gastrointestinal tract was monitored by magnetic marker monitoring (MMM) as a non-invasive imaging method providing information about the tablets' gastrointestinal transit and their in vivo drug release behaviour with very high temporal and spatial resolution [9].

2. Materials and methods

2.1. Magnetic labelling of tablets

A small hole (diameter 1.5 mm, length 2.5 mm) was drilled in both sides (immediate release layer and modified release layer) of Augmentin XR tablets (GlaxoSmithKline, Belgium). Both drill holes were filled with 6 mg ± 1.5 mg of a mixture consisting of 90% black iron oxide (E172, BASF, Germany) and 10% microcrystalline cellulose. The aperture of the drill hole in the extended release layer was closed with magnesium stearate. The tablets were magnetized for 5 min in a homogenous magnetic field of 1 T.

2.2. In vitro dissolution

In vitro standard dissolution experiments were carried out using USP apparatus 2 (USP paddle) at a rotational rate of 50 rpm. For the determination of the relation between the temporal course of the magnetization of the labelled ER tablets and their drug release behaviour a magnetic measurement dissolution tester (MMDT) was used [10]. In the MMDT the magnetized tablets were rotated at a stirring speed of 50 rpm being mounted in a basket and passed a magnetometer in the bottom of the beaker during each rotation. Simultaneously the concentrations of amoxicillin were determined by UV spectroscopy (273 nm) with a fibre optic probe (Cary 50 Scan, Varian, Germany). The dissolution studies were performed in 900 ml phosphate buffer, pH 6.8, USP at 37 ± 0.5 °C. After complete disintegration of the immediate release layer at about 30 min the dissolution medium was renewed in order to avoid disturbances from degradation products of clavulanic acid.

2.3. Study protocol

Nine healthy volunteers (4 females, 5 males, age 26–41 years, body weight 58–90 kg, BMI 21–26 kg/m^2) participated in the three armed randomized study after giving written informed consent. All the protocols were approved by the Charité Berlin ethical committee. The subjects took no medication (with exception of hormonal contraceptives) and abstained from alcohol on study days. On the study days, the tablet was administered together with 240 ml of non-carbonated water in the morning either after at least 10 h fasting, or at the start (after the first bite) of a high carbohydrate standardized breakfast [6], consisting of 2 slices of bread, 40 g cheese, 150 ml orange juice, 150 ml milk and 20 g cereals, or 30 min after a high fat standardized breakfast, consisting of 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 120 ml (four ounces) of hash brown potatoes and 240 ml (eight ounces) of whole milk [5]. The two different compositions of the breakfasts were chosen in order to meet the published experimental data [6] and the information that intake of the meal after a high fat breakfast reduces bioavailability of clavulanic acid [8]. At half-hour intervals starting immediately after ingestion, transport and disintegration of the tablet in the GI-tract was observed by magnetic marker monitoring [9]. Each measurement had a duration of 5 min. The measurements were continued until the tablet was disintegrated to less than 5%. The subjects received 150 ml of non-carbonated water each hour and were allowed to walk around between the magnetic measurements. For lunch, 5 h after tablet administration, a standardized meal was served consisting of chicken fricassee and 240 ml mineral water (non-carbonated). Blood samples were obtained 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480, 510, 540, 570, 600, and 720 min after tablet administration. The samples were centrifuged immediately and stored at −80 °C until analysis of amoxicillin and clavulanic acid.

2.4. Determination of plasma concentrations of amoxicillin and clavulanic acid

The methods were validated according to the most recent US Food and Drug Administration (FDA) guideline on bioanalytical method validation and IBMP system operation procedures. Specificity, linearity, lower limit of quantification (LLOQ), inter-day and intra-day precision and accuracy as well as absolute recovery and stability of amoxicillin and clavulanic acid were evaluated.

Amoxicillin: Plasma samples (0.1 ml) were mixed with 50 μL of the internal standard solution and deproteinized by the addition of acetonitrile. After thorough mixing, the samples were centrifuged for 10 min at 2733g at approximately +4 °C. Fifty microliter of each sample were chromatographed on a reversed-phase column, eluted with an isocratic solvent system consisting of ammonium acetate buffer and acetonitrile and monitored by LC–MS/MS with selected reaction monitoring (SRM).Under these conditions amoxicillin and the internal standard were eluted after approximately 0.8 min. The MacQuan software (version 1.6, PE Sciex, Thornhill, Ontario, Canada, 1991–1998) was used for the evaluation of chromatograms. Drug-free human plasma was used to prepare calibration standards and spiked quality control standards. The lower limit of quantitation (LOQ) during validation was 0.0200 μg/ml. The assay of amoxicillin in human plasma showed an acceptable linearity over a concentration range from 0.0200 to 10.0 μg/ml. The peak area ratios versus theoretical concentrations visually fit well to a straight line. The coefficient of correlation was at least 0.9995. The inter-day precisions of the amoxicillin calibration standards were ⩽6.5% and the relative error was within ±5.6%. The inter-day precision of the spiked quality control standards ranged between 3.6% and 9.2% and the relative error ranged from −4.3% to 6.7%. The intra-day precision and relative error of the amoxicillin assay ranged from 2.1% to 9.1% and between −6.1% and 5.0%. During sample analysis the standard curve in human plasma was linear between 0.0197 and 10.3 μg/ml. The lower limit of quantification for amoxicillin (= lowest calibration point) was 0.0197 μg/ml. The inter-day precision and the analytical recovery of the spiked quality control standards of amoxicillin in human plasma ranged from 4.4% to 6.5% and were 97.0% (19.6 μg/ml, sample was diluted 1:5 with blank human plasma before sample

preparation), 97.6% (8.03 μg/ml), 99.3% (2.50 μg/ml), 96.9% (0.507 μg/ml) and 96.4% (0.0558 μg/ml), respectively.

Clavulanic acid: Plasma samples (0.1 ml) were mixed with 0.1 ml of the internal standard solution and deproteinized by the addition of acetonitrile. After thorough mixing, the samples were centrifuged for 10 min at 2733g at approximately +4 °C. Twenty-five microliter of each sample were chromatographed on a reversed-phase column, eluted with an isocratic solvent system consisting of ammonium acetate buffer and acetonitrile and monitored by LC–MS/MS with selected reaction monitoring (SRM). Under these conditions, clavulanic acid and the internal standard were eluted after approximately 2 min. The MacQuan software (version 1.6, PE Sciex, Thornhill, Ontario, Canada, 1991–1998) was used for evaluation of chromatograms. Drug-free human plasma was used to prepare calibration standards and spiked quality control standards. The lower limit of quantitation (LOQ) during validation was 0.0500 μg/ml. The assay of clavulanic acid in human plasma showed an acceptable linearity over a concentration range from 0.0500 to 8.00 μg/ml. The peak area ratios versus theoretical concentrations visually fit well to a straight line. The coefficient of correlation was at least 0.9997. The inter-day precisions of the clavulanic acid calibration standards were below ⩽4.6% and the relative error was within ±6.5%. The inter-day precision of the spiked quality control standards ranged between 2.7% and 5.4% and the relative error ranged from −1.3% to 6.2%. The intra-day precision and relative error of the clavulanic acid assay ranged from 0.7% to 2.6% and between −0.5% and 6.8%. During sample analysis the standard curve in human plasma was linear between 0.0519 and 8.28 μg/ml. The lower limit of quantification for clavulanic acid (= lowest calibration point) was 0.0519 μg/ml. The inter-day precision and the analytical recovery of the spiked quality control standards of clavulanic acid in human plasma ranged from 3.5% to 6.1% and were 99.4% (7.79 μg/ml), 99.0% (2.42 μg/ml), 96.1% (0.491 μg/ml) and 98.1% (0.0541 μg/ml), respectively.

2.5. Magnetic marker monitoring (MMM)

The volunteers were placed in supine position under a multichannel magnetic field sensor installed in a magnetically shielded room [11]. The measurement device includes 83 SQUID sensors combined electronically to 63 gradiometer channels and a data acquisition system, recording the magnetic field distribution at a sampling rate of 250 Hz. From these measurement data, the location of the ER tablet and the degree of disintegration of the tablets were calculated by fitting the field of a magnetic dipole to the measurement data in inverse field calculation [9]. The locations of the tablets in the GI-tract were determined after transformation to a three-dimensional coordinate system with the origin of the *z*-axis at the back of the volunteer and the origin of the *y*- and *x*-axis at the jugulum of the volunteer. Gastric emptying time was determined either as the exact time point in cases when gastric emptying occurred during the magnetic measurement intervals or, in cases when gastric emptying occurred in the rest phases between two magnetic measurement intervals, as the mean between the final observed time point when the tablet was located in the stomach and the first time point when the tablet was observed in the small intestine. The time point of disintegration of the two magnetic labels was determined by a linear regression of the mean magnetic moments measured for the different time intervals. The first disintegration time (decay 1) reflects the end point of the disintegration process of the immediate release layer. For the second disintegration process (decay 2) two time points are given, indicating the beginning and the end (residual magnetic moment below 5%) of the disintegration of the magnetic label placed in the modified release layer.

2.6. Drug absorption calculation/simulation

Amoxicillin absorption profiles were calculated from the plasma drug concentration profiles by numerical deconvolution (Mathcad 7, Mathsoft Inc., USA). For the calculations, a three-compartment model was applied with the following parameters: V_D = 0.075 l/kg; k_{10} = 0.0478; k_{12} = 0.0108; k_{21} = 0.0108; k_{13} = 0.1132; k_{31} = 0.096 min^{-1} according to published pharmacokinetic data [12].

The values for C_{max} and T_{max} were obtained directly from the measured concentration–time curves. Half-life ($t_{1/2}$) was estimated by log-linear approximation of the terminal data points. AUC_{0-t} was assessed by the trapezoidal rule up to the last sampling time with a concentration above the limit of quantification. $AUC_{0-\infty}$ was calculated by the addition of the extrapolated part after the last sampling time with a concentration above the limit of quantification to AUC_{0-t} using standard techniques. Arithmetic means and standard deviations (SD) are given. Sample statistics was performed with the non-parametric Wilcoxon test. For all pharmacokinetic and statistical evaluations, the SAS 8.0 program package was used (SAS Institute Inc., Cary, USA).

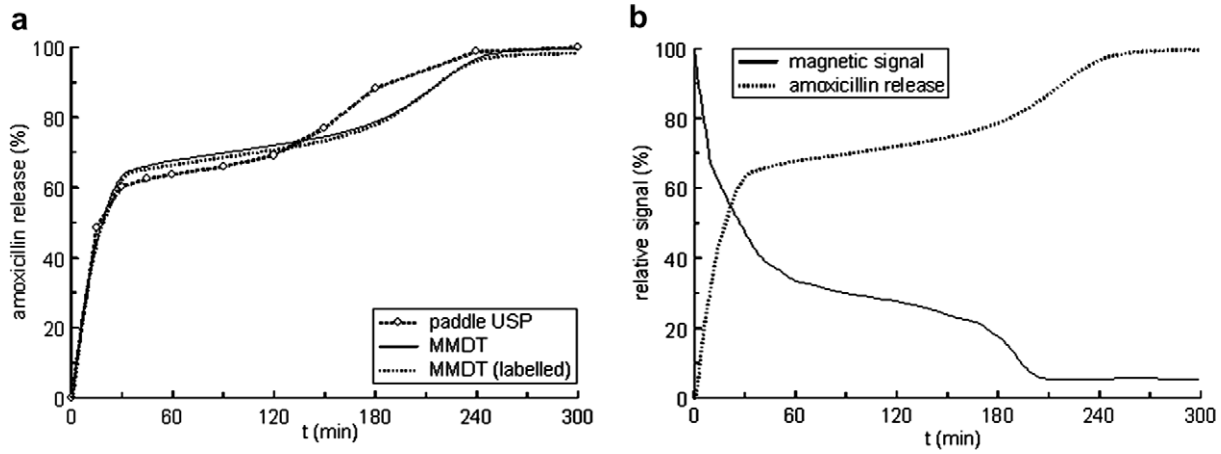

Fig. 1. (a) In vitro amoxicillin dissolution behaviour of original and magnetically labelled and amoxicillin/clavanulate bilayer ER tablets in phosphate buffer pH 6.8 USP in the paddle apparatus according USP (50 rpm, mean, n = 12) and in the magnetic measurement dissolution tester (MMDT, 50 rpm, mean, n = 6); (b) Comparison of the release of amoxicillin and the decrease of the magnetic signal obtained in the MMDT at a stirring rate of 50 rpm (mean, n = 6).

3. Results

3.1. In vitro studies

In the in vitro investigations the ER tablets yielded a triphasic dissolution profile that was not influenced by the magnetic labelling procedure (Fig. 1a and b). The release behaviour was homogenous. The standard deviations of the mean were below 10%. The release profiles can be described as two pulses, an initial rapid burst from the immediate layer and a second delayed pulse from the modified release layer. The time course of the decrease of the magnetic signal of the magnetically labelled tablets determined using the MMDT showed a progression that was well comparable to the release of amoxicillin (Fig. 1b). Accordingly, the disintegration of the two magnetic labels could be applied for the determination of the in vivo release behaviour of the tablets.

3.2. In vivo studies

Following administration in the fasted state gastric emptying of the tablets occurred after a mean residence time in the stomach of 62 min. After ingestion of the tablets at the start of a high carbohydrate breakfast gastric emptying was observed in six subjects, on average, after 222 min. In three volunteers the tablet disintegrated completely in the stomach. After intake of the tablet 30 min after the start of a high fat breakfast gastric emptying was only observed in three subjects, in the other six subjects the tablets had disintegrated before gastric emptying (Table 1). The disintegration of the tablets' immediate release layer was slightly (but not significantly) faster after ingestion with the first bit of a high carbohydrate breakfast compared to ingestion in fasted state and 30 min after the start of a high fat breakfast. The disintegration of the extended release layer was significantly slower after intake together with food compared to fasting conditions (Table 1).

The plasma profiles of amoxicillin and clavulanic acid are shown in Fig. 2. Amoxicillin AUC after administration under fasting conditions was significantly lower than under the two fed conditions whilst there was no difference in AUC between the two intake conditions with food (Table 2). The absorption rates obtained by deconvolution show that the initial absorption derived from amoxicillin release from the immediate release layer (decay 1) was nearly identical under fasting conditions and after tablet intake at the start of the meal (Fig. 3). However, amoxicillin absorption derived from the extended release layer (decay 2) was strongly reduced under fasting conditions compared to fed conditions (Fig. 3). Later T_{max} was observed for both drug substances when the tablet was administered after breakfast. In the case of clavulanic acid this difference is significant. The AUC of clavulanic acid following administration 30 min after the start of a breakfast was significantly reduced compared to the administration under fasting conditions and was also lower than after administration at the start of the breakfast, however, this difference was not significant. The C_{max} 'after breakfast' was significantly lower compared to fasting conditions and intake at the start of breakfast (Table 3).

The influence of tablet administration relative to meal consumption is exemplarily shown in Fig. 4 by means of the localization data obtained in subject 7. Tablet administration at the beginning of the meal resulted in a primary deposit of the tablet close to the pylorus in the distal stomach as it was observed in eight of the nine subjects (Table 1). In contrast, tablet administration after the meal resulted in a primary deposit of the tablet in the proximal stomach, in eight of the nine subjects. In the example shown in Fig. 4a, the tablet administered at the beginning of the meal remained in the distal stomach until gastric emptying. This was not necessarily the case. In four of the eight subjects with

Table 1
Location of initial intragastric deposition (IID), time points of gastric emptying (GE) and decays of the magnetic labels (decay 1 indicates disintegration of the immediate release layer, decay 2 indicates disintegration of the modified release layer) observed after oral administration of a magnetically labelled tablet Augmentin XR under varying administration conditions

Subject	Fasting				At the beginning of a meal				30 min after the meal			
	IID	GE (min)	Decay 1 end (min)	Decay 2 start/end (min)	IID	GE (min)	Decay 1 end (min)	Decay 2 start/end (min)	IID	GE (min)	Decay 1 end (min)	Decay 2 star/end (min)
1	DS	14	16	162/203	DS	–[a]	22	132/189	PS	–[a]	40	175/200
2	PS	185	24	160/207	DS	370	13	329/406	PS	–[a]	11	223/255
3	PS	45	27	174/212	DS	190	25	173/200	DS	–[a]	20	116/150
4	PS	25	17	176/260	DS	210	22	259/293	PS	–[a]	39	278/340
5	DS	160	15	146/180	DS	–[a]	22	266/325	PS	311	63	385/397
6	PS	17	20	114/150	DS	174	20	211/268	PS	290	21	233/280
7	PS	30	46	220/265	DS	195	23	194/255	PS	300	82	281/314
8	PS	35	49	172/270	DS	190	21	175/200	PS	–[a]	16	204/230
9	PS	50	35	205/280	PS	–[a]	23	228/277	PS	–[a]	16	231/260
Mean	–	62	28	170/225	–	222	21	219/268*	–	300	34	236/270*
SD		64	13	31/45		74	3	59/70		11	24	75/74
Median	–	35	24	172/212	–	193	22	211/268	–	300	21	231/260

PS – proximal stomach.
DS – distal stomach.
[a] No gastric emptying observed due to complete tablet disintegration within the stomach.
* $p < 0.05$ (start of decay 2 compared to fasting).

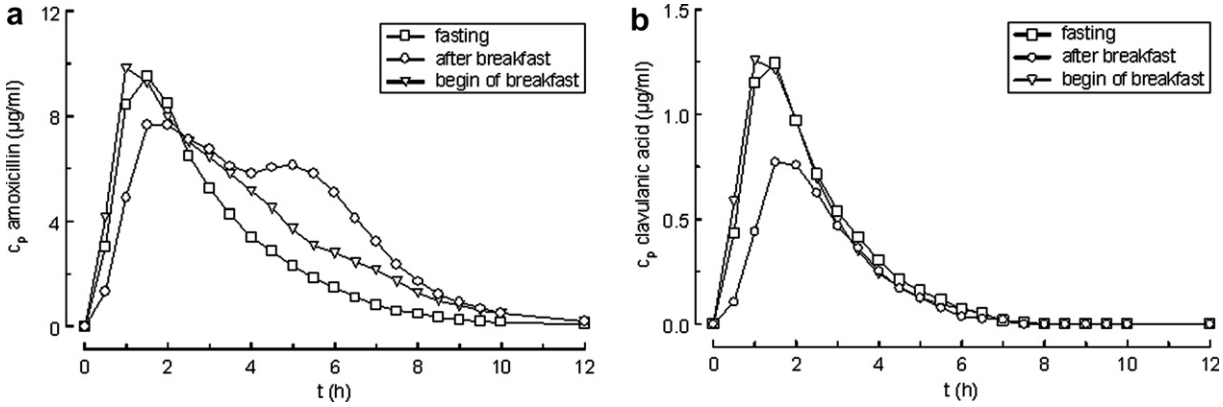

Fig. 2. Plasma concentration profiles (mean, $n = 9$) after administration of amoxicillin/clavanulate bilayer ER tablets under fasting conditions (fasting), at the beginning of a breakfast (begin of breakfast) and 30 min after start of a breakfast (after breakfast); (a) amoxicillin; (b) clavulanic acid.

Table 2
Pharmacokinetic parameters of amoxicillin following oral administration of one magnetically labelled tablet Augmentin XR (1000 mg amoxicillin) under varying administration conditions

	Fasting			At the beginning of a meal			30 min after the meal		
Subject	$AUC_{0\text{-inf}}$[a] (µg min/ml)	T_{max} (min)	C_{max} (µg/ml)	$AUC_{0\text{-inf}}$[a] (µg min/ml)	T_{max} (min)	C_{max} (min)	$AUC_{0\text{-inf}}$[a] (µg min/ml)	T_{max} (min)	C_{max} (min)
1	1506	60	9.0	2261	150	8.3	2629	90	10.2
2	1991	120	9.4	2030	60	9.1	2074	60	7.4
3	1948	90	10.3	2346	90	9.9	2320	90	9.8
4	1706	60	9.9	2670	90	10.6	2526	180	7.9
5	2283	120	11.8	2756	60	9.8	2914	330	11.2
6	1628	60	12.4	2733	60	14.9	2635	90	11.1
7	1633	90	8.9	2033	60	8.7	2353	180	7.4
8	1737	90	10.4	2214	60	11.9	2385	90	9.5
9	2255	120	10.4	3026	120	10.3	3611	120	14.5
Mean	1854	90	10.3	2452*	83	10.4	2605*	137	9.9
SD	280	26	1.2	355	33	2.0	446	84	2.3
Median	1737	90	10.3	2346	60	9.9	2526	90	9.8

[a] Residual area ($AUC_{tlast\text{-inf}}$) contributes in all subjects with less than 2%.
* $p < 0.05$ compared to fasting.

primary tablet deposit in the distal stomach a retrograde intragastric tablet transport into the fundus occurred between 30 and 150 min after tablet administration. In all cases of a primary tablet deposition in the proximal stomach only a slow and stepwise transport of the tablet into the distal stomach was observed as it is illustrated in Fig. 4b.

Gastrointestinal side effects that are likely to be related to the study medication were observed in subject 6 (diarrhoea with nausea and fever) and subject 9 (enterospasms). In both cases the side effects were transient and occurred during the night following tablet intake under fasting conditions. As a further side effect that is likely to be related to the study medication a short episode of headache and nausea was observed during the afternoon following the study medication after breakfast in subject 2.

4. Discussion

The pharmacokinetic study results confirm the data reported by Kaye et al. [6]. They further support the advice that the tablets should be taken at the beginning of a meal as it is recommended in the prescribing information [8]. Under fasting conditions the tablets were emptied from the stomach significantly faster than under fed conditions. This reflects the well known emptying behaviour of the stomach for indigestible solids under fasting and postprandial conditions and is in good accordance with previous studies [13,14]. The reduced gastric emptying of remaining parts of the tablets in case of tablet ingestion after the high fat breakfast compared to tablet ingestion at the beginning of the high carbohydrate breakfast most probably reflects the higher caloric content of the high fat breakfast. The immediate release layer disintegrated within less than 30 min. Consequently, a fast increase in amoxicillin and clavanulate plasma concentration was observed (Fig. 2). The decrease in bioavailability of amoxicillin under fasting conditions was most prominent when gastric emptying of the tablet occurred early and, thereby, the portion of amoxicillin contained in the extended release layer was released within the distal small intestine where it did not result in an increased absorption as determined by deconvolution (Fig. 3a). This finding is in good agreement with the observation that in the deeper small intestine amoxicillin absorption is very poor compared to the upper small intestine [15]. Accordingly, in the two subjects with late gastric emptying of the tablets under fasting conditions (subjects 2 and 5, Table 1) amoxicillin bioavailability was high with at least 95% bioavailability compared to fed conditions in subject 2 and at least 78% in subject 5 (Table 2) and a distinct absorption related to gastric emptying and disintegration of the extended release layer was observed by deconvolution (Fig. 3d).

It has been shown that the peptide transporter PEPT1 is involved in the intestinal absorption of amoxicillin [16]. Consequently, the observed low absorption of amoxicillin from distal parts of the small intestine may result from a decreasing expression of PEPT1 from proximal to distal as it has been reported by

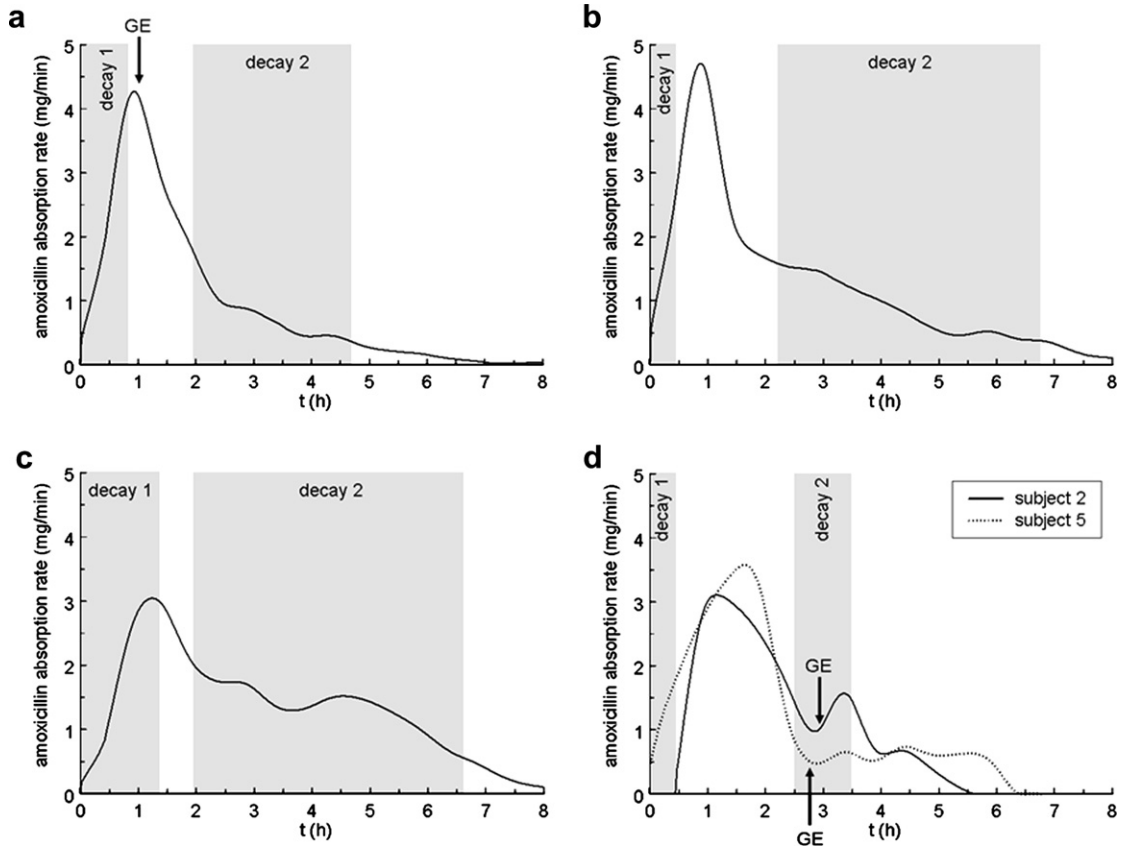

Fig. 3. Amoxicillin absorption rates calculated by deconvolution (lines) and range of disintegration times of the magnetic labels in the immediate release layer (decay 1) and in the extended release layer (decay 2) indicating disintegration (grey areas); (a) mean rate calculated for all subjects after administration under fasting conditions (GE – mean gastric emptying time); (b) mean rate calculated for all subjects after administration at the beginning of a breakfast; (c) mean rate calculated for all subjects after administration 30 min after start of a breakfast; (d) individual absorption rates in subject 2 and subject 5 after administration under fasting conditions, here, the grey areas indicate the range of disintegration times of the two layers observed in the two subjects.

Table 3
Pharmacokinetic parameters of clavulanic acid following oral administration of one magnetically labelled tablet Augmentin XR (62.5 mg clavulanic acid) under varying administration conditions

Subject	Fasting			At the beginning of a meal			30 min after the meal		
	$AUC_{0\text{-}inf}$[a] (μg min/ml)	T_{max} (min)	C_{max} (μg/ml)	$AUC_{0\text{-}inf}$[a] (μg min/ml)	T_{max} (min)	C_{max} (min)	$AUC_{0\text{-}inf}$[a] (μg min/ml)	T_{max} (min)	C_{max} (min)
1	259.9	60	2.24	233.3	90	1.74	210.6	90	1.84
2	148.6	90	1.35	165.9	60	1.38	193.5	90	1.33
3	125.9	90	0.97	152.6	90	1.13	98.9	90	0.82
4	231.2	60	1.58	149.3	60	1.24	49.8	150	0.34
5	156.7	60	1.18	247.8	60	1.96	31.5	150	0.25
6	176.7	90	1.20	193.4	90	1.13	131.5	120	0.83
7	170.3	180	0.81	143.0	120	0.86	74.7	210	0.46
8	234.7	90	1.75	252.5	60	1.94	119.4	90	1.15
9	214.4	90	1.48	165.1	120	1.09	227.3	120	1.72
Mean	190.9	90	1.40	189.2	83	1.39	126.4*	123**	0.97
SD	45.6	37	0.43	44.1	25	0.40	70.9	41	0.58
Median	176.7	90	1.35	165.9	90	1.24	119.4	120	0.83

[a] Residual area ($AUC_{tlast\text{-}inf}$) contributes in all subjects with less than 1%.
* $p < 0.05$ compared to fasting.
** $p < 0.05$ compared to fasting and at the beginning of a meal.

Terada et al. [17]. However, such a gradual decrease in PEPT1 expression along the small intestine was not observed by Englund et al. [18]. They found a steep decrease in PEPT1 expression between the jejunum and the colon. Very poor absorption of amoxicillin from the colon has also been demonstrated by drug administration via intestinal tubing [15]. On the other hand, in our study the extended release layers of all tablets disintegrated before reaching the colon. Therefore, we can exclude that the very poor absorption observed after early gastric emptying is due to the release of amoxicillin directly into the colon. According to human perfusion studies amoxicillin has a low jejunal permeability [19]. In combination with the very poor absorption from colon the low permeability in the small intestine might also act as an absorption window. Thereby, the presented data clearly indicate that there is an absorption window for amoxicillin in the proximal small intestine. However, it remains unclear whether this is due to a decreas-

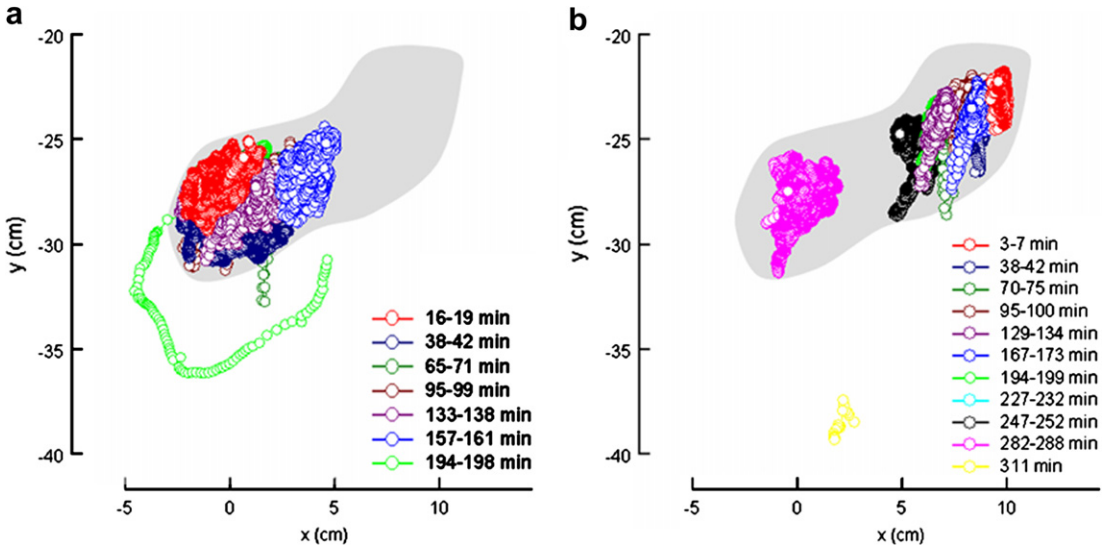

Fig. 4. Frontal view of tablet localisations in subject 7 (temporal resolution 40 ms); (a) tablet administration at the start of breakfast; (b) administration 30 min after start of breakfast. The grey area indicates the stomach.

ing absorption capability along the small intestine or due to low permeability for amoxicillin in the small intestine in combination with very poor absorption from the colon. The anticipation that the intake of amoxicillin and other substrates of PEPT1 together with food will result in a reduced bioavailability due to competition with dietary dipeptides and that therefore such substrates should in general be administered on an empty stomach [16] is not supported by our data.

The reduced bioavailability of clavulanic acid after tablet intake following breakfast is a result of initial deposition of the tablet in the proximal part of the stomach. Consequently, drug release from the immediate release layer occurred under this condition far away from the small intestine as the site of absorption. In contrast, food eaten directly after tablet intake obviously pushed the tablet close to the pylorus. The later T_{max} of amoxicillin and clavulanic acid in the case of tablet intake after breakfast compared to tablet intake at the start of breakfast is a result of this difference. Prolonged gastric residence of dissolved amoxicillin was without consequences for its bioavailability, but unfavourable for clavulanic acid as it is very unstable in aqueous solution, particularly under acidic conditions [20–22]. This understanding is also corroborated by the observation, that under fasting conditions the AUC of clavulanic acid is inversely correlated with the gastric emptying time of the tablet as an indicator for the phase of interdigestive gastric motor activity at the time point of tablet ingestion (Fig. 5a). Furthermore, the AUC of clavulanic acid is decreased when the disintegration of the immediate release layer took place in the proximal stomach compared to disintegration in the distal stomach i.e. closer to the pylorus (Fig. 5b). The bioavailability of amoxicillin was not decreased in the case of long gastric residence times. This finding is in accordance with the observation, that amoxicillin is fairly stable in gastric juice [23]. The different dependency of clavulanic acid and amoxicillin bioavailability on gastric residence time and intragastric site of dissolution also answers the to date open question why the extent of clavulanic acid absorption is in general highly variable and independent on amoxicillin absorption [24].

The study provides a general insight into the role of intragastric deposition of solid dosage forms as a key factor for drug absorption kinetics in combination with food intake. This is of particular relevance for dosage forms with modified drug release characteristics as it was already demonstrated for felodipine ER tablets [13]. In the case of antibiotics pharmacodynamics is closely related to pharmacokinetics. Thereby, the special intake condition "at the beginning of a meal" is essential for effective drug therapy with the investigated ER tablets. Intake under fasting conditions results in reduced amoxicillin absorption and may also cause increased gastrointesti-

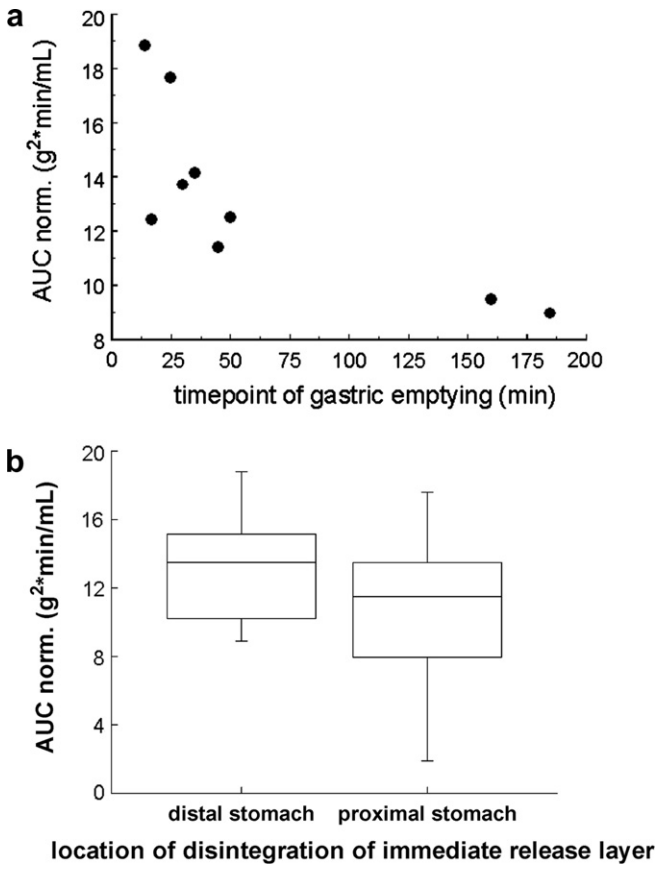

Fig. 5. (a) Relation between AUC of clavulanic acid (normalized for body weight) and gastric emptying time of tablets observed after administration under fasting conditions; (b) box plots of AUC of clavulanic acid (normalized for body weight) under all intake conditions observed after disintegration of the immediate release layer in the proximal stomach (fundus area) and in the distal stomach (corpus/antrum area).

nal side effects like we observed them in two subjects even after one single tablet.

In conclusion, this study demonstrates that in vivo imaging of the behaviour of dosage forms can be very useful to improve drug delivery principles as well as providing information leading to better predictions of in vivo formulation performance. The present data show that amoxicillin is not suitable for extended release delivery unless it is given under conditions that delay gastric emptying because of the absorption window in the upper GI tract. For clavulanic acid it could furthermore be shown, that there is a relationship between gastric residence time, intragastric location of tablet disintegration and reduction of bioavailability, which is very likely to be caused by the instability of drug.

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