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International Journal of Pharmaceutics 335 (2007) 106–113
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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# A new amoxicillin/clavulanate therapeutic system: Preparation, in vitro and pharmacokinetic evaluation

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Received 19 July 2006; received in revised form 27 October 2006; accepted 1 November 2006
Available online 6 November 2006

**Abstract**

A new peroral amoxicillin/clavulanate therapeutic system composed of immediate release tablet and controlled release floating capsule was developed and evaluated by in vivo bioavailability study. Pharmacokinetic (PK) parameters for amoxicillin and clavulanic acid of the new therapeutic systems: AUCt, AUCi, (AUCt/AUCi), Cmax, Tmax, kel, $T_{1/2}$ and additionally for amoxicillin $T_4$ and $T_2$ were calculated from the plasma levels. The study confirmed enhanced pharmacokinetic parameters of a newly developed therapeutic system containing 1500 mg of amoxicillin and 125 mg of clavulanic acid. Prolonged time over MIC of amoxicillin in relation to a regular immediate release amoxicillin/clavulanate formulation was confirmed.


*Keywords:* Amoxicillin; Clavulanate; Therapeutic system; Floating capsule; In vitro study; Pharmacokinetic study

## 1. Introduction

Amoxicillin/clavulanate is a widely prescribed combination of beta-lactam antibiotic and beta-lactamase inhibitor available in different conventional dosage forms for peroral delivery and is considered as a broad-spectrum antibiotic for the treatment of a wide range of bacterial infections, including upper and lower respiratory tract infections and infections of the skin and soft-tissue structures. The clavulanate component inhibits beta-lactamase-mediated inactivation, thereby maintaining amoxicillin's bactericidal activity. This broadens the spectrum of activity of amoxicillin to include amoxicillin-sensitive and beta-lactamase-producing (amoxicillin resistant) bacterial strains. This antibiotic is formulated in various ratios of the broad-spectrum penicillin amoxicillin and the beta-lactamase inhibitor clavulanate potassium, available worldwide in a wide range of parenteral (IV) and peroral formulations that meet the treatment needs of adult and pediatric patients in the general-practice and hospital institutions. The drug is well tolerated. In Europe, the daily parenteral (IV) therapeutic dose in adults ranges up to 12 g/1200 mg amoxicillin/clavulanate (Dollery, 1999a,b; Todd and Benfield, 1990).

Differences in extent of amoxicillin absorption from various regions of the gastrointestinal tract have been determined. Amoxicillin absorption is rate and site dependent in the gastrointestinal tract. The drug is well absorbed in the duodenum and jejunum, with no significant differences in absorption when administered as a bolus or 4-h infusion, but absorption is decreased and rate dependent in the ileum, where more drug is absorbed as an infusion compared to bolus. Amoxicillin is unabsorbed when infused in all colonic regions (Barr et al., 1994).

There have been several attempts to improve the bioavailability of amoxicillin in order to reduce the number of dosings per day, i.e. increase the dosing intervals.

Taking into account the fact that amoxicillin is mainly absorbed from the upper small intestine the bioavailabilty of amoxicillin cannot be improved by conventional sustained release formulations such as polymer matrix tablet formulations (Lek, unpublished data). Therefore, more innovative approaches must be considered to retain the dosage form at the absorption site.

Over the last three decades, various approaches have been pursued to increase the retention of an oral dosage form in the stomach (Singh and Kim, 2000), including floating systems (Deshpande et al., 1997), swelling and expanding systems (Urquhart and Theeuwes, 1984; Mamajek and Moyer, 1980), bioadhesive systems (Alvisi et al., 1996; Lenaerts and Gurny, 1990; Lehr, 1994; Ponchel and Irache, 1998; Wilson and

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doi:10.1016/j.ijpharm.2006.11.007

Washington, 1989), modified-shape systems (Cargill et al., 1988; Caldwell et al., 1988a,b,c; Fix et al., 1993; Kedzierewicz et al., 1999), high-density systems (Rednick and Tucker, 1970; Bechgaard and Ladefoged, 1978; Davis et al., 1986), and other delayed gastric emptying devices (Gröning and Heun, 1984, 1989).

Other delayed gastric emptying approaches of interest include sham feeding of indigestible polymers (Russell and Bass, 1985a,b; Leung et al., 1993) or fatty acid salts (Gröning and Heun, 1984, 1989) that change the motility pattern of the stomach to a fed state, thereby decreasing the gastric emptying rate and permitting considerable prolongation of drug release.

The idea of this investigation was to develop a peroral therapeutic system including immediate release (IR) amoxicillin/clavulanate dosage form and controlled release (CR) floating amoxicillin dosage form that would expand the time over MIC of amoxicillin in vivo in relation to a regular IR amoxicillin/clavulanate formulation in the form of a coated tablet, thereby obtaining all the advantages of continuous intravenous infusion while avoiding the limitations of the parenteral application.

A new pharmacokinetically enhanced peroral therapeutic system was developed to provide more effective therapy against organisms with increasing resistance to amoxicillin, notably *Streptococcus pneumoniae*. The development of a therapeutic system took into account the characteristic gastrointestinal (GI) absorption properties of amoxicillin with major absorption in upper small intestine and poor colonic absorption (Barr et al., 1994) and non-linear absorption kinetics (Torres-Molina et al., 1992).

Failure to eradicate the infecting organism can lead to the development of resistant clones (Dagan et al., 2001) and it was found that there was no regrowth of cultures after modelling the higher amoxicillin/clavulanic acid dose (White et al., 2004; DeRyke et al., 2006). It has been shown that higher doses of amoxicillin/clavulanic acid can increase the rate of eradication and that bacterial eradication is associated with an improved clinical cure. Optimising pharmacodynamic principles therefore prevents the emergence of resistance (White et al., 2004; Dagan et al., 2001; DeRyke et al., 2006). The new pharmacokinetically enhanced formulations were developed to achieve amoxicillin concentrations in plasma that will exceed 4 μg/mL for at least 35–40% of the dosing interval. This provides complete cover for pneumococcal strains with intermediate penicillin resistance (i.e. penicillin MICs of 0.5–<2.0 μg/mL) which is equivalent to the intermediate resistance breakpoint for amoxicillin of 4 μg/mL.

## 2. Materials and methods

### 2.1. Preparation of therapeutic system

#### 2.1.1. Amoxicillin floating capsule

Hard capsules made of hydroxypropylmethylcellulose, Vcaps size #00, natural transparent V001/V001 (Capsugel, France) were used. Empty capsule bodies were film coated with a suspension of Surelease (Colorcon, UK): hydroxypropylmethylcellulose Methocel E6 (Colorcon, UK) 60:40 (sample 011A001A) or 70:30 (sample 011A001B) in a perforated coating pan Labcoat 1 System (O'Hara, Canada) to apply the dry coat in amount of 6.5 mg/cm$^2$.

The mixture of 80 g amoxicillin trihydrate (Sandoz, Spain), 9.5 g hydroxypropylmethylcellulose Methocel K100LV (Colorcon, UK), 9.5 g Avicel PH102 (FMC, USA) and 1.0 g magnesium stearate (Faci, Italy) was homogeneously blended (mixture M) and the tablets were prepared by direct compression using a laboratory press fitted with a 7.4 mm concave faced punch and die set. Tablets of 180 mg weight and hardness of 6–10 kP were prepared (tablet T).

Five hundred and sixty milligrams of the same mixture M was manually filled into each coated capsule body and the 180 mg amoxicillin tablet (tablet T) was added on top of the mixture and the capsule was closed. Each capsule (011A001A or 011A001B) contained 603 mg amoxicillin trihydrate (equivalent to 500 mg amoxicillin).

The schematic presentation of the coated floating capsule is presented in Fig. 1. The function of a tablet is to fix the granulate in the capsule body to retain the same structure of the granulate during production, transport and administration. Low soluble coating is used to enable floating of the content of the capsule which starts to hydrate and swell after the uncoated capsule cap is dissolved and water penetrates into the tablet and granulate in the capsule body.

Two therapeutic systems, i.e. therapeutic systems A and B were developed and their bioavailability was tested in vivo.

The therapeutic system A (1500 mg of amoxicillin and 125 mg of clavulanic acid) included one IR Amoksiklav® 625 mg tablet and two floating capsules 011A001A.

The therapeutic system B (1500 mg of amoxicillin and 125 mg of clavulanic acid) included one IR Amoksiklav® 625 mg tablet and two floating capsules 011A001B.

#### 2.1.2. Amoxicillin/clavulanate IR tablet

Conventional amoxicillin/clavulanic acid (Amoksiklav® 625 mg) including 500 mg of amoxicillin in the form of amoxicillin trihydrate and 125 mg of clavulanic acid in the form of potassium clavulanate were taken from a regular production batch produced and released for the market.

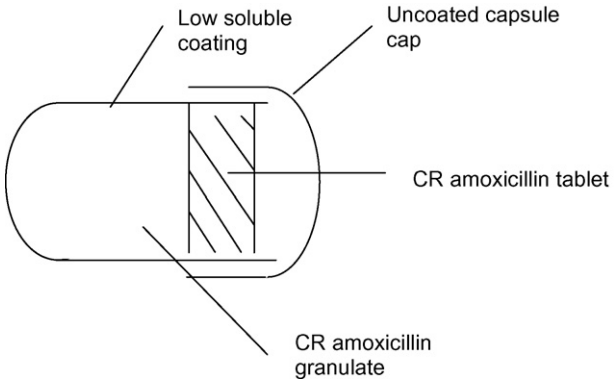


Fig. 1. Schematic presentation of the coated floating capsule.

### 2.2. In vitro floating test

Floating test was performed in 0.001 M HCl and phosphate buffer pH 4.5 at 37 °C. Four hundred milliliters of the medium in each vessel was heated on a 10 station magnetic stirrer to 37 °C and mixed at 50 rpm. One floating capsule was placed to each vessel on the surface of the medium; stirring at 50 rpm was maintained. Capsules were observed for 5 h every 1/2 h for changes in the formulation and floating characteristics. Remarks were written when significant changes were observed.

### 2.3. In vitro dissolution studies

The dissolution rates of amoxicillin from the floating capsule were monitored using a dissolution tester according to the USP paddle method—Apparatus 2 (Vankel VK7010, USA), under the following conditions: rotation speed 75 rpm; release medium 900 mL phosphate buffer pH 4.5, maintained at 37 °C. Offline automatic sampling with Vankel VK8000 system was used.

Amoxicillin levels in dissolution media were monitored spectrophotometrically (Shimadzu UV160A spectrophotometer, Japan) at 272 nm. Dissolution studies of six capsules were performed and the average values were calculated.

The dissolution rates of amoxicillin and clavulanic acid from therapeutic system (one IR Amoksiklav® 625 mg tablet and two floating capsules) were monitored using a dissolution tester according to the USP paddle method—Apparatus 2 (Vankel VK7010, USA), under the following conditions: rotation speed 75 rpm; release medium 900 mL phosphate buffer pH 4.5, maintained at 37 °C. Offline automatic sampling with Vankel VK8000 system was used.

Amoxicillin and clavulanic acid levels in dissolution media were monitored by HPLC at 272 nm. Dissolution studies of six parallels were performed and the average values were calculated.

### 2.4. Bioavailability study

The objective of the study was to determine bioavailability of two new amoxicillin/clavulanate therapeutic systems versus the standard Amoksiklav® 625 mg tablet (500 mg amoxicillin and 125 mg clavulanate) (Lek Pharmaceuticals d.d., Slovenia) under fed conditions.

Twelve healthy male volunteers, aged 18–45, participated in the in vivo evaluation. All gave informed consent to participation and the study protocol was approved by the Ethic Committee at the Slovenian Ministry of Health. The study was conducted in a randomized, single dose, three period, three formulations, and six sequence cross-over design under fed conditions. The wash out period was 1 week.

Subjects were requested to abstain from alcohol, to avoid xanthine and grapefruit containing food or beverages (i.e. tea, coffee, cola drinks, or chocolate) for 48 h before and during each study period. Subjects were informed not to take any drugs for at least 14 days before and during the study. The medications used in a study were:

- Therapeutic system A (1500 mg of amoxicillin and 125 mg of clavulanic acid) included one IR Amoksiklav® 625 mg tablet and two floating capsules 011A001A.
- Therapeutic system B (1500 mg of amoxicillin and 125 mg of clavulanic acid) included one IR Amoksiklav® 625 mg tablet and two floating capsules 011A001B.
- Formulation C (500 mg of amoxicillin and 125 mg of clavulanic acid): one IR Amoksiklav® 625 mg tablet.

After an overnight fast and 30 min before their scheduled dosing times, subjects were received standard high fat breakfast. A standardized light lunch and dinner were served at 12:00 and 6:00 p.m. A sandwich was given to the volunteers between 8:00 and 9:00 p.m. The composition of meals was identical for all three periods.

At the scheduled dosing times, each volunteer was given a peroral dose of the assigned formulation, with 240 mL of water, according to the randomization list. To facilitate the blood sampling, slight delays in drug ingestion were made and sampling times were adjusted accordingly. Blood sampling schedule was: just before drug intake and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14 and 24 h after drug administration (22 samples per subject per period). The subjects remained at the clinical site until 24 h after drug administration. The same procedure was used for all periods.

### 2.5. Analytical procedure of biological samples

The tubes with blood samples were centrifuged for 10 min at 3500 rpm under refrigeration. Plasma samples were stored at −70 °C until analysis.

#### 2.5.1. Amoxicillin

Plasma samples for amoxicillin determination were prepared by deproteinization with perchloric acid and extraction with 1,2-dichloroethane. Fifty microliters of 60% $HClO_4$ was added to 1.0 mL of plasma sample and shaken on a vortex. After that 1.0 mL of dichloroethane was added and shaken once again. After centrifugation the clear upper layer was used for injecting in the HPLC system.

Amoxicillin in plasma was analysed by HPLC on RP column with UV detection at 240 nm. The mobile phase consisted of buffer (pH 7.0) and methanol in a ratio of 100:4.5 (v/v).

The method was specific and sensitive, with a quantifiable limit of 0.2 μg/mL and calibration range 0.2–30.0 μg/mL. Within-run precision was in the range 2.3–2.7% and accuracy 88.4–96.1. Between-run precision (five consecutive days) was in the range 5.6–6.4% and accuracy 93.6–94.6.

#### 2.5.2. Clavulanic acid

Plasma samples for clavulanic acid determination were prepared by extraction and derivatization with triazole. 1.0 mL of dichloroethane was added to 1.0 mL of plasma sample and shaken on a vortex. After centrifugation the 0.5 mL of triazole derivatizing reagent was added to 0.5 mL of the upper layer. The content was shaken on a vortex and heated on a water bath at

30 °C. After that, the solution was prepared for injecting in the HPLC system.

Clavulanic acid in plasma was analysed by HPLC on RP column with UV detection at 317 nm. The mobile phase consisted of 400 mL of methanol per 4000 mL of phosphate buffer. The method was specific and sensitive, with a quantifiable limit of 0.1 μg/mL and calibration range 0.2–30.0 μg/mL. Within-run precision was in the range 3.0–3.2% and accuracy 88.5–95.8. Between-run precision (five consecutive days) was in the range 4.8–6.5% and accuracy 91.7–101.4.

### 2.6. Pharmacokinetic analysis

The plasma levels of the amoxicillin and clavulanic acid were used to establish the pharmacokinetic profile of tested formulations. The following pharmacokinetic parameters were calculated: AUCt, AUCi, (AUCt/AUCi), Cmax, Tmax, kel, $T_{1/2}$ and additionally for amoxicillin $T_4$ and $T_2$. The explanation of PK parameters is as follows:

- AUCt: the area under the plasma concentration versus time curve, from time 0 to the last measurable concentration, calculated according to the trapezoidal method.
- AUCi: the AUCi was calculated by adding Cp/kel to the AUCt, where Cp is the plasma concentration achieved at the time where the last concentration occurred which is above lower limit of quantitation (LQCT) and kel is the corresponding elimination rate constant.
- AUCt/AUCi: the ratio of AUCt to AUCi.
- Cmax: maximum observed plasma concentration.
- Tmax: time of the maximum observed plasma concentration.
- kel: terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve. The parameter was calculated by linear least-squares regression analysis using the maximum number of points in the terminal log-linear phase.
- $T_{1/2}$: terminal elimination half-life was calculated as 0.693/kel.
- $T_4$: the period of time during which amoxicillin plasma concentrations were $\geq$MIC = 4 μg/mL.
- $T_2$: the period of time during which amoxicillin plasma concentrations were $\geq$MIC = 2 μg/mL.

PK parameters were estimated using Kinetica software (InnaPhase Corporation, Copyright© 1997–2006). For amoxicillin the time above the minimum inhibitory plasma concentration ($T_4$, $T_2$) was calculated manually by graphical interpolation, for each volunteer separately.

### 2.7. Statistical analysis

Descriptive analysis including the mean, standard deviation (S.D.) and coefficient of variation (CV) was used to describe the pharmacokinetic parameters.

Pharmacokinetic parameters were analysed by analysis of variance (ANOVA) for a cross-over design to determine statistically significant ($p < 0.05$) differences between treatments, sequences of treatments, subject nested within sequence, and periods of administration. The statistical analysis was performed using SAS® and PROC GLM procedure for the analysis of variance. Each ANOVA included calculation of least-squares means (LSM), differences between formulation LSM, and the standard error associated with these differences. ANOVA was carried out on the logarithmically transformed AUCt, AUCi and Cmax data. (Note for Guidance on the Investigation of Bioavailability and Bioequivalence, 2002).

The analyses included all bioavailability data from all subjects who regularly completed the study.

## 3. Results and discussion

Over the years, several formulations of amoxicillin/clavulanic acid were developed and the ratio of amoxicillin to clavulanic acid has been varied to reflect prescribing needs, to improve patients' convenience, and also as a response to new prescribing guidelines for the treatment of more severe infections of those caused by resistant microorganisms. However, in the majority of peroral formulations the unit dose of clavulanic acid remained at 125 mg for adults. In several years the amount of amoxicillin in amoxicillin/clavulanic acid formulations increased to ratio of up to 16:1.

Zero order kinetics and a selective zone of absorption in the upper gastrointestinal tract has been suggested (Barr et al., 1994; Vree et al., 2003). So the aim of our study was to develop and evaluate a new therapeutic system which would provide immediate release of an initial dose of 500 mg of amoxicillin and all clavulanic acid (125 mg) and sustained release of remaining 1000 mg of amoxicillin. With this strategy, a more sustained plasma profile of amoxicillin than that obtained with conventional formulations can be achieved and therefore the time drug levels exceed the MIC ($T >$ MIC) is prolonged. The sustained release and absorption is achieved by a floating capsule containing amoxicillin and retarding excipients which remains floating in the stomach for a prolonged period of time.

Both formulations—floating capsules with coating A and B and containing 500 mg of amoxicillin were tested for floating time in 0.001 M HCl and phosphate buffer pH 4.5 at 37 °C. Capsule floating was observed for 5 h and notes were made every half hour if any changes were observed. As expected from the coating formulation floating capsule 011A001A starts to break, swell and hydrate faster that the floating capsule 011A001B.

In 0.001 M HCl sample 011A001A starts to hydrate within 1 h and is floating until 4 h when half of the matrix is drowned to the bottom. In 5 h all the matrix is drowned. Sample 011A001B hydrates within 2 h and remains floating for 5 h.

The processes of capsule floating, hydration and erosion are similar in phosphate buffer pH 4.5 but occur about 2 h earlier due to better solubility of the capsule coating in the phosphate buffer, i.e. sample 011A001A is drowned in 3.5 h and sample 011A001B is floating in the middle of the vessel after 5 h, but erosion is significant.

The dissolution of the floating capsules was performed in phosphate buffer pH 4.5, maintained at 37 °C. Due to different composition of the capsule coating the dissolution of amoxicillin

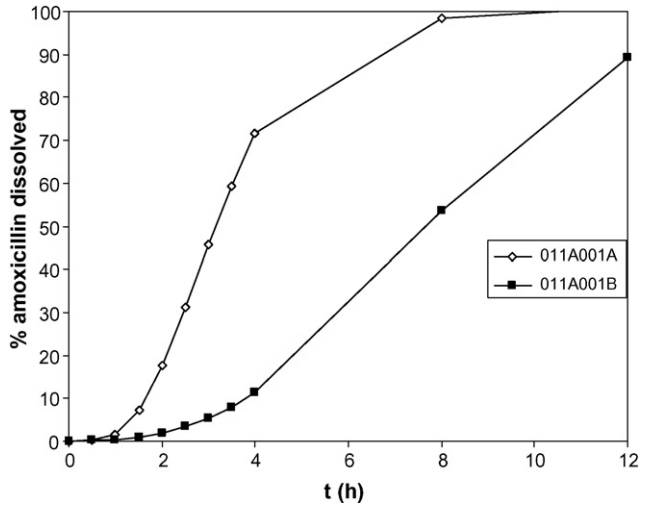


Fig. 2. Dissolution rates of amoxicillin from the floating capsule 011A001A and 011A001B in phosphate buffer pH 4.5 ($n = 6$).

from the coated capsule 011A001A is faster than amoxicillin dissolution from the coated capsule 011A001B (Fig. 2) what is in correlation to higher ratio of insoluble ethylcellulose to soluble hydroxypropylmethylcellulose in sample 011A001B (ratio 70/30) than in sample 011A001A (ratio 60/40).

A biphasic dissolution profile was determined for the dissolution of amoxicillin from the therapeutic system A or B, i.e. amoxicillin from IR tablet was dissolved immediately and amoxicillin release from the floating capsule was sustained with a different dissolution profile for systems A and B (Fig. 3). Amoxicillin dissolution from therapeutic system B is more sustained due to lower solubility of the capsule coating (higher ethylcellulose ratio in the coating suspension).

Amoxicillin from the IR tablet represents the same initial dose in the PK study for both therapeutic systems as well as formulation C and amoxicillin from the floating capsules provides

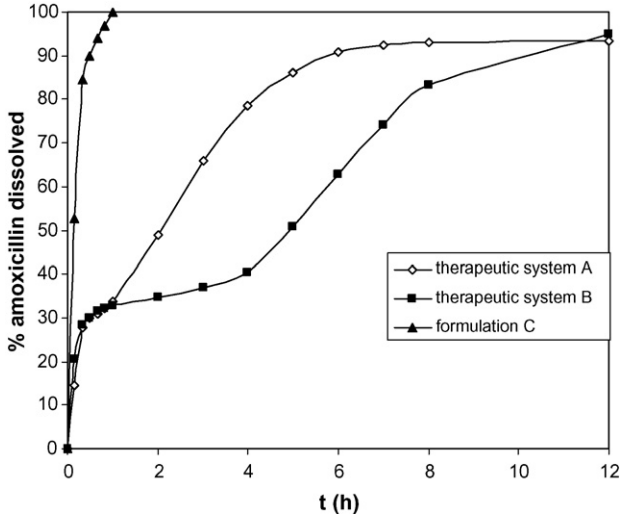


Fig. 3. Dissolution rates of amoxicillin from therapeutic system A (1500 mg), therapeutic system B (1500 mg) and formulation C (500 mg) in phosphate buffer pH 4.5 ($n = 6$).

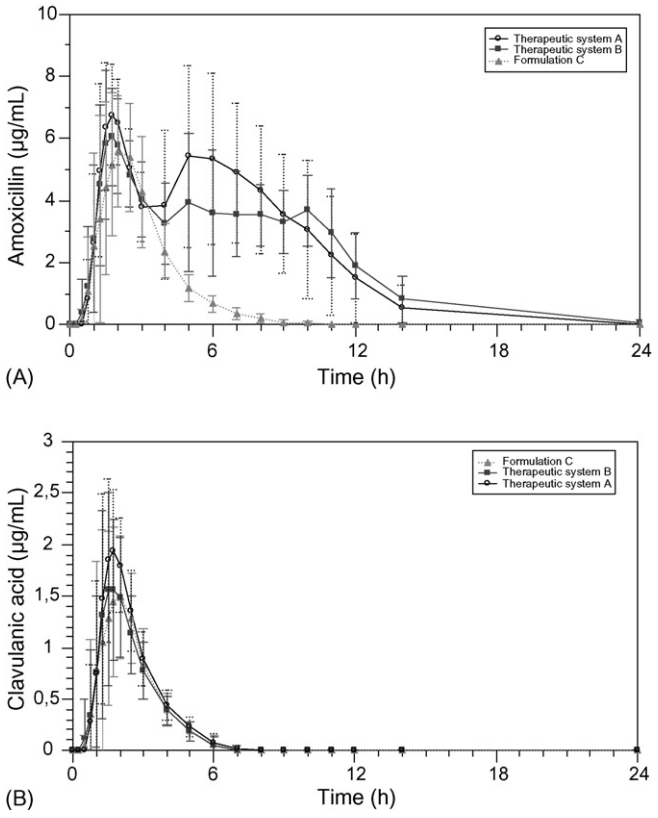


Fig. 4. Mean plasma concentrations for amoxicillin (A) and clavulanic acid (B) for therapeutic system A, therapeutic system B and formulation C (PK study with 12 male volunteers).

sustained prolonged plasma concentrations of amoxicillin from therapeutic systems A and B.

Conventional Amoksiklav 625 mg tablet (500 mg of amoxicillin and 125 mg of clavulanic acid) served as initial dose of amoxicillin and all clavulanic acid and was the same in all three systems evaluated in vivo. All clavulanic acid from this immediate release tablet is dissolved in 20 min.

Mean plasma amoxicillin concentrations obtained in a bioavailability study are presented in Fig. 4A. It is evident that both new therapeutic systems (A and B) have prolonged absorption in comparison with a standard Amoksiklav® 625 mg tablet (formulation C).

Since the amoxicillin dose in therapeutic systems A and B was 1500 mg compared to 500 mg in formulation C, it was expected that amoxicillin AUCt of therapeutic systems A and B equals to the AUCt of a standard Amoksiklav tablet (formulation C) multiplied by 3. This ratio is actually almost 3 for a therapeutic system A (the ratio A/C is 2.9), for a therapeutic system B more drug remains unabsorbed (the ratio B/C is 2.7) (Table 1).

Tmax of amoxicillin, calculated separately for floating capsules in the therapeutic systems A and B, are 6.61 and 6.52 h, respectively. Ratio of amoxicillin Cmax, calculated separately for both floating capsules in the therapeutic systems A and B and Cmax of therapeutic systems A and B, are 0.88 for A and 0.73 for B. These results proved that delayed, sustained and substantial amoxicillin absorption was achieved with floating capsules.

Published studies have indicated that prior exposure to clavulanic acid causes a prolonged inhibition of the beta-lactamase, thus suggesting that although clavulanic acid levels may be undetectable protection may still be afforded to amoxicillin due to a recovery period which prevents regrowth (Thorburn et al., 1996). For beta-lactams, the duration of time when plasma concentrations of drug exceed MIC is the crucial parameter that determines their efficacy (Craig, 1998; Sourgens, 2004). These findings support the fact that once the beta-lactamase is inhibited the pharmacodynamics of action of the combination is reflected by the $T>$MIC of amoxicillin (Thorburn et al., 1996; Vree et al., 2003; Bronner et al., 2001). Therefore, a daily clavulanic acid dose of 125 mg b.i.d. (e.g. as administered with most amoxicillin/clavulanate formulations twice daily regimen) is fully justified in order to inhibit beta-lactamases and there is no need for further dose increases.

Clavulanic acid is incorporated in the immediate release part of both therapeutic systems A and B and formulation C and PK parameters are similar for all three formulations tested (Fig. 4B). The ratios of pharmacokinetic parameters for therapeutic systems A and B compared to formulation C are presented in Table 1.

It can be deduced from experimental as well as clinical studies that there is a minimal effective time, which needs to be covered by inhibitory beta-lactam concentrations at the site of infection in order to achieve cure. Studies have indicated that for amoxicillin/clavulanic acid a $T>$MIC of 35–40% of the dosing interval is predictive of high bacteriological efficacy (Craig, 1998; Woodnutt and Berry, 1999a,b; Andes and Craig, 1998; DeRyke et al., 2006). For pneumococcal infections, regimens which gave rise to a $T>$MIC for amoxicillin for 34% of a 24-h dosing period resulted in significant reductions in the number of viable bacteria, however, maximum bacteriological cure was obtained when $T>$MIC was 35–40% (Woodnutt and Berry, 1999b). New therapeutic systems were developed to improve the therapeutic efficacy of amoxicillin. The time that serum levels remain above the MIC is termed the PK/PD index and it has been shown to correlate with the efficacy of beta-lactam treatment (Andes and Craig, 2002; Frimodt-Moller, 2002; White et al., 2004; DeRyke et al., 2006). Mean time of amoxicillin plasma concentrations above MIC 2 and MIC 4, achieved with all three formulations tested in a bioavailability study is presented in Table 2.

Table 1
Mean values and ratios of geometric means (arithmetic means for Tmax) of amoxicillin and clavulanic acid pharmacokinetic parameters

| | Cmax (μg/mL) | Tmax (h) | AUCt (μg/mL h) | AUCi (μg/mL h) |
|---|---|---|---|---|
| Amoxicillin | | | | |
| Mean A | 8.27 | 4.25 | 48.16 | 49.75 |
| Mean B | 7.08 | 2.52 | 44.52 | 46.81 |
| Mean C | 7.17 | 1.89 | 16.75 | 17.23 |
| Ratio A/B | 1.17 | 1.59 | 1.08 | 1.06 |
| Ratio A/C | 1.15 | 1.97 | 2.88 | 2.89 |
| Ratio B/C | 0.99 | 1.24 | 2.66 | 2.72 |
| Clavulanic acid | | | | |
| Mean A | 2.10 | 1.67 | 4.07 | 4.29 |
| Mean B | 1.61 | 1.85 | 3.29 | 3.53 |
| Mean C | 1.78 | 1.82 | 3.27 | 3.51 |
| Ratio A/B | 1.30 | 0.90 | 1.24 | 1.22 |
| Ratio A/C | 1.18 | 0.94 | 1.25 | 1.22 |
| Ratio B/C | 0.91 | 1.04 | 1.01 | 1.01 |

Table 2
Mean time of amoxicillin plasma concentrations above MIC 2 and 4 for therapeutic systems A and B and formulation C

| | A | B | C |
|---|---|---|---|
| $T_2$ (h) (% of 12 h dosing interval) | 9.28 (77%) | 10.64 (89%) | 3.04 (25%) |
| $T_4$ (h) (% of 12 h dosing interval) | 6.05 (50%) | 3.81 (32%) | 1.74 (15%) |

The results show that for therapeutic system A, the time above MIC 4 is long enough to provide high bacteriological efficacy with twice daily dosing. Therapeutic system A achieved higher plasma concentrations in comparison with therapeutic system B and longer time above MIC 4. The impact of floating capsules in both therapeutic systems is significant. The contribution of IR part of the formulation in both therapeutic systems to the time that plasma levels remain above the MIC is 1.74 h for MIC 4 and 3.04 h for MIC 2. Consequently the contribution of two floating capsules of the therapeutic system A to the time that plasma levels remain above the MIC is about 4 h for MIC 4 and about 6 h for MIC 2. This is more than we could expect for 1000 mg IR dose (two immediate release 500 mg tablets of amoxicillin), while the bioavailability of amoxicillin is dose dependent (Westphal et al., 1991) and significant non-linearity of absorption occurs above 776 mg doses (Chulavatnatol and Charles, 1994). For amoxicillin, an absorption window in the upper small intestine exists. The drug is well absorbed in the duodenum and jejunum, but absorption is decreased and rate dependent in the ileum. Amoxicillin is unabsorbed when infused in all colonic regions (Barr et al., 1994). The non-linearity of the gastrointestinal absorption of amoxicillin was also a result of a study on six healthy subjects, performed by Paintaud et al. (1992). They observed dose dependency of the extent of amoxicillin absorption, with a lower than expected mean maximum plasma concentration (49%) and fraction of the dose absorbed (39%) after the 3 g dose calculated from the 500 mg dose, assuming kinetic linearity. The dose dependency of amoxicillin absorption was confirmed by a trend to an increased time of absorption for the high dose (Paintaud et al., 1992). According to the literature, amoxicillin pharmacokinetics was also modelled using a two-compartment disposition model and a saturable time-constrained absorption model with a storage compartment. Simulations performed over a wide dose range (50–10,000 mg) demonstrated that the fraction absorbed decreases non-linearly from 90% at 50 mg to 22% at 10,000 mg (Piotrovskij et al., 1994). Westphal et al. (1991) investigated the absorption kinetics of amoxicillin in humans using the Loo-Rigelman method. The results showed evidence of a saturable carrier-mediated uptake of amoxicillin. The dipeptide carrier system would appear to be the most likely transport mechanism (Westphal et al., 1991).

For drugs that exhibit an absorption window and saturable absorption mechanism, the increase of a dose has a limited effect

on plasma concentrations if a drug is not retained in the GIT before the absorption window. The new therapeutic systems are designed in a way to increase the bioavailability of amoxicillin formulations with doses above 776 mg.

GlaxoSmithKline developed a formulation containing 2000 mg of amoxicillin and 125 mg of clavulanic acid, designed to combat infections caused by *S. pneumoniae*, including penicillin-resistant isolates with amoxicillin MICs of up to 4 μg/mL. An efficacy analysis on five clinical trials was conducted in adult patients with community-acquired pneumonia. The formulation achieved high rates of success in patients who had *S. pneumoniae* infection and serological evidence of atypical infection. Its safety profile was similar to that of conventional formulations (File et al., 2005).

The results of therapeutic system A are comparable with the $T>$MIC for an amoxicillin MIC of 4 μg/mL for the pharmacokinetically enhanced amoxicillin/clavulanic acid 2000/125 mg b.i.d. dose. The $T>$MIC for an amoxicillin MIC of 4 μg/mL for the therapeutic system A with 1500/125 mg dose was 50% of 12 h dosing interval. According to the literature, the percent of dosing interval when plasma concentrations are above MIC 4 was 49% for the pharmacokinetically enhanced amoxicillin/clavulanic acid 2000/125 mg b.i.d. dose and 41% for the 1000/125 mg dose t.i.d. (White et al., 2004; File et al., 2005). Time above MIC 4 μg/mL was 3.3 h or 27.5% for b.i.d. dose for amoxicillin/clavulanic acid 875/125 mg Solutab® dispersible tablet and also for a conventional Augmentin® 875/125 mg tablet. The appropriateness of using b.i.d. dosage regimen for amoxicillin/clavulanic acid (875/125 mg) dispersible tablets was described in the literature (Sourgens et al., 2004). They calculated various time above MIC needed when b.i.d. dosing regimen would be given and the corresponding plasma concentration of amoxicillin for four important pathogens: *S. aureus*, *Haemophilus influenzae*, *M. catarrhalis* and *S. pneumoniae*. The obtained pharmacodynamic breakpoints were compared with defined NCCLS (National Committee for Clinical Laboratory Standards) breakpoints. The calculated PD breakpoints were in good agreement with NCCLS breakpoints except for *H. influenzae* (Sourgens et al., 2004). If we compare the new therapeutic system with the results above, the $T>$MIC for an amoxicillin MIC of 4 μg/mL was 6 h for our therapeutic system A. This is long enough to meet all NCCLS breakpoints for above mentioned pathogens including that for *H. influenzae*.

Based on the time that plasma levels remain above the MIC, bioavailability study confirmed enhanced efficacy for newly developed therapeutic system containing 1500 mg of amoxicillin and 125 mg of clavulanic acid when compared to amoxicillin/clavulanic acid 875/125 mg tablet b.i.d. and similar efficacy to amoxicillin/clavulanic acid 2000/125 mg b.i.d. dose.

Irrespective of the higher dose of amoxicillin and known non-linear absorption kinetics above 776 mg of amoxicillin, no significant loss of amoxicillin was confirmed for the new therapeutic system where the controlled release of amoxicillin is provided by a gastroretentive floating capsule. Higher doses or improved bioavailability of antimicrobials could overcome their inefficacy due to the increased resistance to common antimicrobials. The developed therapeutic system uses both possibilities in such a way that the bioavailability is improved irrespective to increased dose of amoxicillin.

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