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ORIGINAL ARTICLE

In vivo–in vitro correlation for amoxicillin trihydrate 1000 mg dispersible tablet

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Abstract

Objectives: An in vivo–in vitro correlation (IVIVC) for amoxicillin dispersible tablet was established. *Methods*: The model of absorption was constructed with type 2 dissolution apparatus and with the use of variable hydrodynamic conditions simulating passage through upper part of gastrointestinal tract. The in vivo test was performed on 24 volunteers. *Summary of results*: The predicted errors for AUC and C_{max} were calculated. Conditions of the in vitro test were discriminative to capsules, which were claimed to have lower bioavailability. The zero-order process of amoxicillin absorption enabled to establish the IVIVC more easily. *Overall conclusions*: The described model showed features of level A correlation for immediate release dosage form and the zero-order process of amoxicillin absorption is an advantage for establishing IVIVC.

Key words: *Amoxicillin; dispersible tablets; dissolution; in vivo–in vitro correlation; IVIVC; model of absorption*

Introduction

Amoxicillin is a semisynthetic aminopenicillin widely used in chemotherapy worldwide. An earlier study showed that amoxicillin like other β-lactam antibiotics has a specific zero-order absorption mechanism via a saturated carrier-mediated pathway[1]. It takes place mainly in the upper part of the small intestine; hence, it is important for oral formulations to provide a rapid dissolution of the active substance. Cortvriendt[2] showed that amoxicillin in the form of a capsule is poorly absorbed (about 15–20%) than amoxicillin in the form of dispersible tablet or oral suspension where the absorption is almost complete. Such a difference is not significant from an efficacy point of view, but may be important when the side effect and the influence of unabsorbed part of the drug on the intestinal bacterial flora are taken into consideration.

Amoxicillin is poorly soluble in water, but in the form of dispersible tablet has a high extent of absorption from the gastrointestinal (GI) tract; hence, it can be classified biopharmaceutically as class II drug. In such a case developing in vivo–in vitro correlation (IVIVC) is possible. Most of the described IVIVCs for various drugs are concerned with prolonged release forms, because it is relatively difficult to develop such correlations for immediate release products. The specific absorption mechanism of amoxicillin enabled to establish IVIVC of the dispersible tablets. The positive slope of plasma concentration–time curve is mostly linear and it is not absolutely necessary to take all the points from the absorption phase for the dissolution model. The critical points of the model are: residence time in the stomach, which is claimed to be about 30 minutes and the T_{max} (1.75 hours), where the absorption rapidly slows down. The overall transit time through small intestine lies between 3 and 5 hours, but studies by Barr[3] showed that the absorption of amoxicillin is highly efficient only in duodenum and jejunum, which are approximately 60% of the length of whole small intestine; hence, the absorption effective transit time is about 2 hours. The results taken from Wagner–Nelson (WN) absorption profile[4] are similar showing that at T_{max} most of the amoxicillin is absorbed.

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A reliable IVIVC can be an effective tool to establish dissolution specification and to justify exemption from bioavailability studies in case of post-approval changes, like variations in formulation, changing production scale etc.

The aim of the work was to establish IVIVC for a 1000 mg amoxicillin dispersible tablet. The way of developing such a correlation for immediate release dosage form with a zero-order absorption process is going to be designed.

Materials and methods

Batches used in the study

Three batches of commercially available dispersible tablets (Amotaks® Dis produced by Polfa Tarchomin SA, Warsaw, Poland) containing 1000 mg of amoxicillin (as trihydrate) were used in the study. One batch was a bioavailability batch (batch A). In vitro dissolution tests for IVIVC were carried out with other two batches (batches B and C). All the batches came from the same validated production process. Additionally, one batch of commercially available capsules (Amotaks® produced by Polfa Tarchomin SA, Warsaw, Poland) containing 500 mg amoxicillin (as trihydrate) was used in the dissolution study (two capsules in one vessel with the use of EP sinkers (Pharma Test Apparatebau GmbH, Hainburg, Germany)) to show that the developed IVIVC model for immediate release amoxicillin dispersible tablet can be discriminative against capsules of amoxicillin, which are claimed to have a lower bioavailability.

In vitro dissolution testing

Dissolution testing was performed using Pharma test PTWS 3 (Pharma Test Apparatebau GmbH, Hainburg, Germany) USP and EP apparatus with 2 (paddles) operating at 25 and 85 rpm, respectively. Water was used as the dissolution medium. Approximately 10 mL samples were withdrawn from each dissolution vessel at 2 minutes 30 seconds, 8 minutes 46 seconds, 15 minutes, 25 minutes, 35 minutes, 60 minutes, and filtered prior to spectrophotometric analysis on Unicam UV3 spectrophotometer (Unicam Ltd., Cambridge, UK) at wavelength equaled 272 nm. The analytical method used was a validated procedure used for routine dissolution control of production batches. Each dissolution profile was taken from 12 units. The coefficient of variation for all the points was less than 10%. Variable hydrodynamic conditions were implemented in the study to imitate a minimal absorption of the drug in stomach. During the initial 2 minutes 30 seconds of the experiment the paddles operated at 25 rpm and after that the rotation increased to 85 rpm simulating the rapid absorption of amoxicillin in the duodenum and the proximal part of the small intestine.

In vivo studies

Study design

Twenty-four adult volunteers (12 males and 12 females) between the ages of 19 and 40 years (mean 23.9 ± 5.6) and weighing between 52 and 82 kg (mean 64.4 ± 9.1), with body mass index (BMI) between 20.1 and 24.9 (mean 21.9 ± 1.6) were empanelled for this study. All subjects fulfilled criteria of inclusion and exclusion for the test. Each subject signed a written informed consent prior to study participation. The last meal was served on the day before drug administration after which overnight fast was maintained. In the morning, each subject received one 1000 mg tablet with 250 mL of water. The first meal on the day of the study was served 4 hours after start of the experiment. The blood samples were drawn immediately prior to drug administration (0 hour) and then at 0.25, 0.5, 0.75, 1, 1.5, 1.75, 2, 2.5, 3, 4, 5, 7, 9, and 12 hours after dosing. Plasma samples were centrifuged and frozen. Amoxicillin plasma concentrations were measured by high-performance liquid chromatography (HPLC) method with linearity in the range of 0.025–25,000 μg/mL on the Varian ProStar 9000 apparatus (Varian Inc., Palo Alto, CA, USA).

Pharmacokinetic analysis

The model-independent method was used for pharmacokinetic analysis. C_{max} and T_{max} were the observed values. K_e was calculated from the negative of the slope of the log-linear part of the plasma concentration–time curve using linear regression. The area under the plasma concentration–time curve from time 0 to 12 hours, AUC_{0-12}, was calculated using trapezoidal rule and extrapolated to infinity by the following equation:

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_{12\,h}}{K_e} \quad (1)$$

where $C_{12\,h}$ is the estimated concentration at time t = 12 hours and $AUC_{0-\infty}$ thearea under the plasma concentration–time curve from time zero to infinity.

The absorption profile of amoxicillin was calculated using the WN[4] equation:

$$\frac{A_t}{A_\infty} = \frac{C_t + K_e \times AUC_{0-t}}{K_e \times AUC_{0-\infty}}. \quad (2)$$

The results of WN equation were expressed as percentage of the dose absorbed.

In vitro dissolution data were converted into plasma concentration–time curve data using the following equation:

$$C_{t+1} = \frac{2 \times \Delta F \times K_e \times AUC_{0-\infty} + C_t \times (2 - K_e \times \Delta t)}{2 + K_e \times \Delta t}, \quad (3)$$

where C_{t+1} is the plasma concentration at $t+1$, $\Delta F = F_{t+1} - F_t$ the difference of amount dissolved between t and $t+1$, and C_t the calculated plasma concentration at time t, $\Delta t = t_{t+1} - t_t$.

IVIVC data analysis

In the study, mean pharmacokinetic and mean dissolution rate data were used to establish the correlation. U.S. Food and Drug Administration (FDA)[5] criteria of predicted error were used to determine level of IVIVC. Moreover, the correlation coefficient was established. The concentration–time data from bioavailability study were transformed into in vivo absorption profile using WN equation. After that, in vitro absorption model was built using variable hydrodynamic conditions to imitate different absorption rates in the various parts of GI tract. A time scale factor of 0.0833 (12 times) was used to enable comparison of in vivo and in vitro data.

The dissolution data were transformed into predicted plasma concentration–time curve with Equation (3) and the PE for AUC and C_{max} was calculated using the following equation:

$$\%\mathrm{PE} = \frac{|\mathrm{Obs.} - \mathrm{Pred.}|}{\mathrm{Obs.}} \times 100. \quad (4)$$

The correlation coefficient was calculated using Microsoft Excel.

Results and discussion

Mathematical analysis

The results of the in vivo study and the WN plot of percentage of the dose absorbed versus time are shown in Figures 1 and 2, respectively. The mean pharmacokinetic parameters are shown in Table 1.

The results of dissolution tests for a 1000 mg dispersible tablets and 2 ¥ 500 mg capsules are shown in Figure 3. The results of conversion of the dissolution data into predicted plasma concentration–time curve by Equation (3) are shown in Figure 4. The comparison of observed and predicted AUC_{0-12} and C_{max} with PE calculations are shown in Table 2.

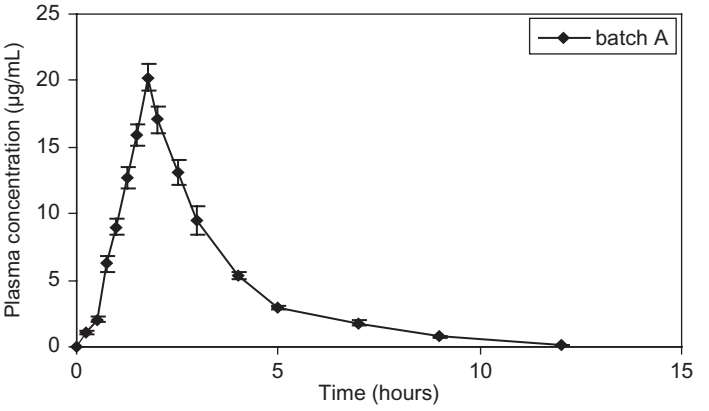

Figure 1. The results of the in vivo study.

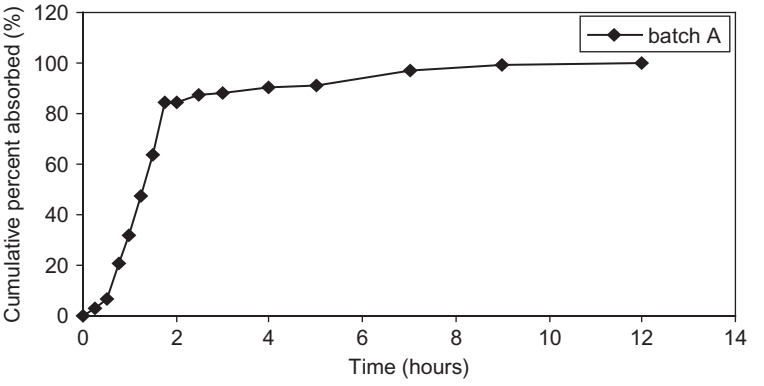

Figure 2. Wagner–Nelson plot of percentage of the dose absorbed versus time.

Table 1. Mean pharmacokinetic parameters of amoxicillin.

AUC_{0-12} (mcg mL/h)	$AUC_{0-\infty}$ (mcg mL/h)	K_e (h^{-1})	C_{max} (mcg/mL)	T_{max} (hours)
52.28	52.52	0.676	20.214	1.75

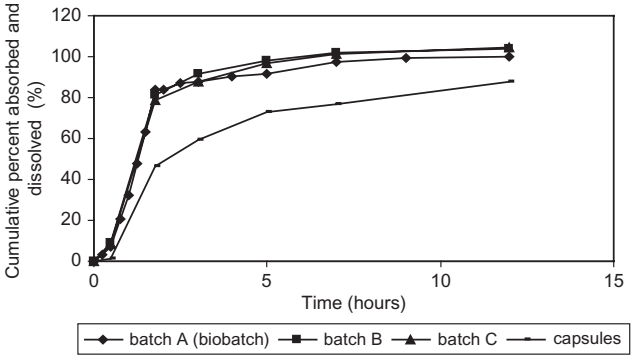

Figure 3. The results of dissolution tests for a 1000 mg dispersible tablets and 2 × 500 mg capsules and Wagner–Nelson plot of percentage of the dose absorbed versus time (batch A).

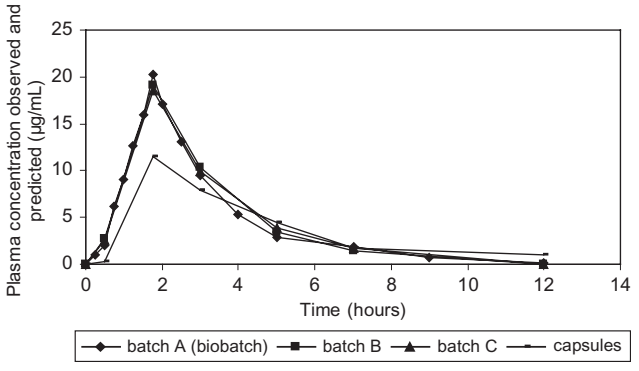

Figure 4. The results of conversion of the dissolution data into predicted plasma concentration–time curve by Equation (3) and the results of the in vivo study (batch A).

In vitro dissolution model

The construction of in vitro absorption model for amoxicillin dispersible tablet is based on the several important time points from the GI transit of the product. At the beginning, a minimal absorption during stomach residence (30 minutes, 2 minutes 30 seconds factored) is simulated with low rotation speed of paddles. Increasing rotation speed after this time point simulates the rapid zero-order absorption of amoxicillin during transit through duodenum and jejunum. As the dissolution time of immediate release dosage form is relatively short, 12 times factor was used to establish the correlation. The absorption of amoxicillin is a zero-order process; the initial parts of bioavailability and WN curves are linear between 0.5 hour (2 minutes 30 seconds factored) and 1.75 hours – T_{max} (8 minutes 46 seconds factored). That fact is considered to be very useful for building an in vitro model of immediate release form with zero-order absorption process, because only two points (linear part of the curve) are necessary to simulate absorption during the dissolution test.

From descending (nonlinear) part of bioavailability curve points at 3 hours (15 minutes), 5 hours (25 minutes), 7 hours (35 minutes), and 12 hours (60 minutes) were used. Decreasing the quantity of points in the in vitro model makes dissolution procedure easier and limits changes in the volume of dissolution medium, what may effect the solubility of the amoxicillin. Although amoxicillin is not absolutely stable in gastric juice having a decomposition rate of 4.5%/h[6], water was chosen as the dissolution medium (USP medium). The decomposition rate is too small to have impact on the results and the changing pH of the medium during the test seems to be unnecessary and makes the procedure too complicated.

C_{max} results calculated from Equation (3) are lower than C_{max} observed during bioavailability studies, although they fulfill the FDA criteria of PE. It is possible to have predicted C_{max} values closer to real ones by increasing the rotation speed of paddles, but it makes the model less discriminative to batches having lower extent of bioavailability. From a clinical point of view for antibiotics more important is AUC because of the time when the plasma concentration is higher than minimal inhibitory concentration (MIC).

Table 2. Observed and predicted AUC_{0-12} and C_{max} with predicted error (PE) calculations and correlation coefficient.

Property	C_{max}	AUC_{0-12}	PE AUC	PE C_{max}	Correlation coefficient
Dispersible tablets (µg/mL), batch A	20.214	52.28	—	—	—
WN transformed data for batch A (%)	84.16	—	—	—	—
Dispersible tablets predicted in vivo data (µg/mL), batch B	19.138	55.199	5.58	5.32	0.9949
Dissolved (%), batch B	81.08	—	—	—	0.9978
Dispersible tablets predicted in vivo data (µg/mL), batch C	18.563	55.043	5.28	8.17	0.9949
Dissolved (%), batch C	78.46	—	—	—	0.9965
Capsules predicted in vivo data (µg/mL)	11.434	44.74	14.43	43.44	0.9472
Dissolved (%) capsules	46.25	—	—	—	0.9614

Criteria of in vivo–in vitro correlation

The reliability criteria of IVIVC were described by FDA[5] guidance. It takes into consideration only extended release formulations, so to estimate the level of correlation only the average absolute percent prediction error criteria for AUC and C_{max} were used. The results less than 10% reflect level A correlation. There is no defined criterion for immediate release product.

In our case, we used two batches of the amoxicillin dispersible tablets for establishing the correlation versus bioavailability batch. Moreover, to show that the model is discriminative to forms that have lower bioavailability dissolution test of the capsules was performed. The results were similar to literature data describing difference in bioavailability between dispersible tablets and capsules. The difference in AUC in the model proposed was 14.44% compared to a value of 18.65% taken from the literature for 500 mg dose of amoxicillin[2].

Conclusions

The results obtained show a possibility to establish reliable IVIVC for amoxicillin dispersible tablets. It is also important that the described model shows features of level A correlation for immediate release dosage form, which is rather rarely spotted in literature. The zero-order process of amoxicillin absorption is an advantage for establishing IVIVC.

Declaration of interest: The authors report no conflicts of interest.

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