Eur J Clin Pharmacol (1992) 43: 283–288

European Journal of Clinical Pharmacology
© Springer-Verlag 1992

Nonlinearity of amoxicillin absorption kinetics in human

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Received: November 22, 1991/Accepted in revised form: March 30, 1992

Summary. Specialised gastrointestinal absorption of amoxicillin has been suggested in man and has been demonstrated in animals. In order to study the rate and extent of amoxicillin absorption, six healthy subjects were given 500 mg IV and two oral doses (500 mg and 3 g as a suspension). Absorption kinetics was analysed by compartmental modelling, noncompartmental methods and by calculation of absorption rates using deconvolution.

Dose-dependency of the extent of amoxicillin absorption was observed, 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. Zero-order kinetics of absorption was apparent in some subjects after the 500 mg dose, both from model fitting and absorption rate profile. However, no pattern consistent with pure first-order or zero-order absorption was observed after both oral doses in any individual. The dose-dependency of amoxicillin absorption was confirmed by a trend to an increased time of absorption for the high dose.

The results show the variable nature and nonlinearity of the gastrointestinal absorption of amoxicillin and indicate the involvement of a number of factors, in addition to simple diffusion.

Key words: Amoxicillin; bioavailability, pharmacokinetics, intestinal absorption

The problem of getting a variety of hydrophilic drugs, e.g. certain antibiotics and chemotherapeutics, absorbed through the gut wall justifies continuing pharmacokinetic and mechanistic studies. Experiments utilising rat intestinal pouches have shown that the absorption of amoxicillin and of several other aminopenicillins is capacity limited [1–4] and competitive [3]. Accordingly, specialised absorption of amoxicillin in the gut wall must be presumed.

In man, extensive absorption of this widely used antibiotic is observed at low doses, despite its ionised state at intestinal pH [2]. Dose-dependent bioavailability has been shown, with a progressive decrease in the dose normalised area under the curve and urinary recovery, after the oral intake of four different doses ranging from 375 mg to 3 g [5]. Also, the concomitant administration of a high dose of cyclacillin, another aminopenicillin, delayed the time to the peak plasma concentration of amoxicillin and decreased the fraction absorbed [6].

The saturation of amoxicillin absorption is of practical significance, since, for example, the 3 g dose recommended as prophylaxis against endocarditis in dental surgery [7], is higher than the dose that will be 50% absorbed [5]. The fraction of the amoxicillin dose not absorbed causes ecological disturbances of the intestinal microflora [8]. Moreover, if specialised or active absorption of amoxicillin were confirmed, other interactions at the level of absorption might be anticipated. A detailed analysis of the rate of absorption has now been made after the administration of an intravenous (500 mg) and two oral doses (500 mg and 3 g) of amoxicillin to 6 healthy subjects.

Subjects and methods

Subjects

Six healthy subjects, 4 m and 2 f, participated after routine clinical examination and laboratory tests. None had a history of allergy to penicillins or cephalosporins, and RAST (penicillin G and penicillin V specific IgE) was negative. They had taken no drugs for at least 1 week before the study, with the exception of an oral contraceptive by 1 female. None of the subjects smoked.

Study design

The subjects were given amoxicillin as 500 mg IV and oral doses of 500 mg and 3 g. The subjects received the IV dose first, followed by the oral doses in randomised order, with a minimum interval of 1 week. Immediately before the IV administration, 500 mg amoxicillin sodium (Beecham Pharmaceuticals) was dissolved in 10 ml sterile water (total volume 10.4 ml). Venflon® i.v. cannulae were inserted into each forearm. The 500 mg dose was given using an infusion pump (Syringe Infusion Pump 22, Harvard Apparatus, South Na-

Table 1. Results of the model fitting of plasma concentrations after oral amoxicillin 500 mg and 3 g. Plasma concentrations were best predicted by a first-order (1) or zero-order (0) rate of absorption model, or were not satisfactorily predicted by either (N)

Subjects	500 mg	3 g
1	1	1
2	0	0
3	1	N
4	0	N
5	N	N
6	0	N

tick, Mass.) over exactly 5 min (2.08 ml · min^{-1}). The infusion fluid was analysed for the amoxicillin concentration. Oral doses were given as a freshly prepared 50 mg · ml^{-1} suspension of amoxicillin trihydrate, corresponding to 500 mg or 3 g (granulate for suspension, Imacillin®, Astra Läkemedel AB, Sweden). Water was added to give a total volume of 150 ml in both cases.

Study procedures

The subjects fasted for a minimum of 8 h (from midnight to drug dosing), with the exception of 250 ml water taken upon rising at least 1 h before dosing. Throughout all the drug administrations they drank a total of 150 ml water. Then, the subjects continued fasting for 3 h after drug intake, except for 100 ml water taken after 1, 2, 4 and 5 h, and thereafter permitted *ad libitum*. A standardised lunch, consisting of meat pie and mixed vegetables, with 250 ml apple juice, was served 3 h after dosing. Subjects lay on a bed until lunch and did not engage in any strenuous activity during the rest of the day. Blood samples 3 ml were collected in heparinised tubes just before dosing and 0, 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150 min and 3, 4, 5, 6, 7, 8, 9, 10 h after it. Plasma was quickly separated by centrifugation for 7 min at 2000 g. The samples were placed at −20°C within 15 min of collection and were stored at −70°C after each experiment until assayed. Informed consent was obtained from all the subjects and the study was approved by the Ethics Committee of Huddinge University Hospital.

Amoxicillin assay

Amoxicillin was determined by ion-pair reversed phase HPLC after purification by solid phase extraction [9]. The standard curves, obtained by analysis of plasma solutions of amoxicillin (5 concentrations), were linear between 0 and 125 mg · l^{-1} ($r = 0.999$). The minimum detectable concentration was 0.1 mg · l^{-1}. The coefficient of variation was 2.6% at 40 mg · l^{-1} ($n = 19$) and 2.5% at 1 mg · l^{-1} ($n = 10$).

Pharmacokinetic analysis

The absorption of amoxicillin was analysed both by compartmental and noncompartmental methods. Adjustment to the administered dose was made according to the assay. The difference between the intended and actual doses was less than 10%.

The compartmental modelling employed weighted least squares fitting to the plasma concentrations by use of the nonlinear regression program NLIN (SAS Institute). Weights inversely proportional to predicted concentrations were used [10]. Conventional equations were used for all the models [11]. A two-compartment model was fitted to the IV data [12], allowing for the effect of the duration of the infusion. One-compartment models with either first-order or zero-order absorption were fitted to the individual oral data. A delay of absorption (t_{lag}) was incorporated in the models. Two-compartment models with either first-order or zero-order absorption were applied as well, but were rejected as no biexponential decline was apparent. For noncompartmental analysis, the area under the plasma concentration versus time curve (AUC) and the area under the first moment curve (AUMC) were measured by the combination of the linear trapezoidal method for the first part of the curve and the logarithmic trapezoidal method in the exponential declining phase [13]. Half-life ($t_{1/2}$) and terminal disposition constant (λ_z) were calculated from log-linear regression of the terminal phase. The extrapolation of AUC and AUMC from the last sampling time to infinity employed the last measured concentration and λ_z [13]. The mean percentages of AUC and AUMC obtained by extrapolation beyond the last data point were 1.0% and 5.9%, respectively. Plasma clearance (CL) and volume of distribution at steady state using AUMC (V_{ss}) were calculated after IV administration, and so were the fraction of the dose absorbed (f), mean residence time (MRT) and mean absorption time (MAT) [11].

The profile of the absorption rate was also analysed both by the Loo-Riegelman method and the deconvolution method described by Iga [14]. For the Loo-Riegelman method, the constants k_{10}, k_{21} and k_{12}, obtained from the two-compartment disposition model fitted to the IV data, were used. After estimation of the fraction absorbed to time t (f_a), the apparent rate of absorption ($\Delta Xa/\Delta t$, in mg · h^{-1}) was obtained as [11, 15]:

$$\frac{\Delta Xa}{\Delta t} = f \cdot D \cdot \frac{f_a(t_{i+1}) - f_a(t_i)}{t_{i+1} - t_i}$$

where Xa is the amount absorbed, D is the oral dose and $f_a(t_{i+1}) - f_a(t_i)$ is the difference between the percentages absorbed at two consecutive sampling times t_i and t_{i+1}. The rate of absorption was plotted against the midpoint of each interval, $(t_i + t_{i+1})/2$. The rate of absorption obtained by the deconvolution method was expressed in mg · h^{-1} by multiplying the value obtained (fraction of dose · h^{-1}) by D, and the cumulative amount absorbed with time was calculated.

Statistical analysis

The suitability of the weighting factor used in the nonlinear regression (1/predicted concentration) was verified by plotting the weighted residuals against time and against the predicted concentrations [16]. The results of the fitting to the absorption models were analysed by visual inspection, meeting of the convergence criteria, and comparison of the residual sums of squares. Pharmacokinetic parameters of the two oral doses were compared by analysis of variance and the existence of an order effect was ruled out. 90% confidence intervals (CI) were calculated according to Schuirmann [17]. The Wilcoxon test was used to compare t_{max}. The difference was considered significant at $P < 0.05$. The results are expressed as mean and (SD), unless otherwise stated.

Results

A two-compartment disposition model was applicable after all IV doses. As shown in Table 1, there was inter- as well as intraindividual variability in the results of the fitting of the plasma concentrations after the oral doses. The concentrations in one subject after 500 mg and in 4 subjects after 3 g could not satisfactorily be fitted to either the first-order or the zero-order model.

The calculated pharmacokinetic parameters are listed in Table 2. The increase in peak plasma concentration (C_{max}) and the increase in AUC with dose from 500 mg to 3 g were 49% (range 36–59) and 39% (CI 27–48) less than proportional to dose assuming first-order kinetics. Accordingly, the fraction of dose absorbed (f) was 39%

Table 2. Pharmacokinetic parameters obtained by noncompartmental analysis after administration of 500 mg IV and 500 mg and 3 g oral doses of amoxicillin to 6 healthy subjects

	500 mg IV	500 mg orally	3 g orally	Significance
AUC ($mg \cdot h \cdot l^{-1}$)	34 (6)	25 (3)	91 (17)	
			147 (18)[a]	$P < 0.003$[b]
C_{max} ($mg \cdot l^{-1}$)		8.8 (1.8)	26.8 (3.9)	
			52.6 (10.9)[a]	$P < 0.003$[b]
f		0.72 (0.09)	0.45 (0.11)	$P < 0.003$[c]
t_{max} (h)[e]		1.375 (0.75–2.5)	2 (1.5–3)	NS[c]
MRT (h)	1.31 (0.11)	2.62 (0.63)	2.91 (0.42)	NS[c]
MAT (h)		1.31 (0.64)	1.60 (0.46)	NS[c]
$t_{1/2}$ (h)	1.81 (0.49)	1.23 (0.27)	1.27 (0.14)	NS[d]
CL ($ml \cdot min^{-1}$)	248 (38)			
V_{ss} (l)	18.4 (2.8)			

[a] Calculated from the value after the 500 mg oral dose, assuming linearity; [b] Between observed and calculated value after the 3 g dose; [c] Between the 2 oral doses; [d] Between the 3 doses; Values are expressed as mean (SD), except [e]: median (range). Abbreviations are described in "Methods"

lower after 3 g compared to 500 mg. Mean plasma concentrations after the two oral doses are shown in Fig. 1. The time to peak concentration (t_{max}) was longer after 3 g than 500 mg in 5 of the 6 subjects. The MAT was longer in 5 of the 6 subjects (range 9–77 %) after 3 g than after 500 mg. The mean ratio of MAT 3 g/MAT 500 mg was 131 % (CI 98–176). Furthermore, 2 of the 6 subjects after 500 mg and 5 after 3 g had a MAT longer than MRT_{iv} (range 33–81 % and 10–90 %, respectively), suggesting that amoxicillin followed absorption-limited kinetics (a "flip-flop" model) in those subjects. The half-life did not differ between the 500 mg and the 3 g oral doses, but it tended to be longer after 3 g than 500 mg IV.

The results of the Loo-Riegelman and deconvolution methods were similar, and the curves of the cumulative amount absorbed, calculated from the results of the Iga method, were no different from the curves obtained directly by the Loo-Riegelman method. As the rate profile of the Iga deconvolution method was somewhat smoother, the Iga method was chosen for analysis of the rate of absorption vs. time. The individual absorption rate profiles are shown in Fig. 2, and the data are also displayed as cumulative amount and fraction absorbed with time (Fig. 3). Inter- as well as intraindividual variability was observed both in the maximum rate of absorption and in the time course of the rate of absorption.

Discussion

The study has confirmed the dose-relationship of the extent of absorption of amoxicillin in man, as previously reported [5]. A capacity-limited absorption process has been shown in animal models [1, 3, 4]. Such studies have often used solutions of amoxicillin and other amino-β-lactam antibiotics introduced into prepared intestinal pouches in situ. The disappearance of drug has been found to follow mixed Michaelis-Menten and first-order kinetics [4, 18]. In those animal models, degradation, solubility limitation [2, 4], gastric emptying and intestinal transit time, as well as differences along the intestinal tract, could not influence the kinetics of absorption.

Three approaches have been used in the present study to assess the input function of amoxycillin into the body after oral administration. A pharmacokinetic model with a first-order absorption rate was fitted to individual data sets. The procedure did not succeed, as only 3 out of 12 plasma concentration curves were satisfactorily described by the model. A zero-order absorption rate model gave a better result only in some of the other experiments (Table 1). Furthermore, the pattern seemed dose-related in half of the subjects. Thus, the absorption of amoxicillin appears too irregular to be generally described by a simple first-order or zero-order process. By contrast, a successful fit of a two-compartment disposition model to the IV data was obtained, confirming the previous report on the stability of the disposition of amoxicillin [19].

Second, mean residence time parameters were studied. As expected, a longer MAT after 3 g than after 500 mg was observed in the 5 subjects who showed a later t_{max}. The observation of one outlier in 6 subjects illustrates the inherent variability of amoxicillin absorption. As 5 of the 6 subjects had an MAT after 3 g that was longer than MRT_{iv}, absorption-limited kinetics can even be foreseen in those

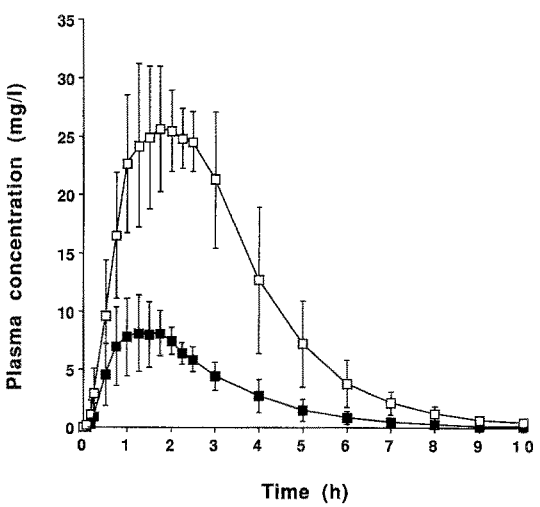

Fig. 1. Mean (SD) plasma concentrations of amoxicillin in 6 healthy subjects after 500 mg (■) and 3 g (□)

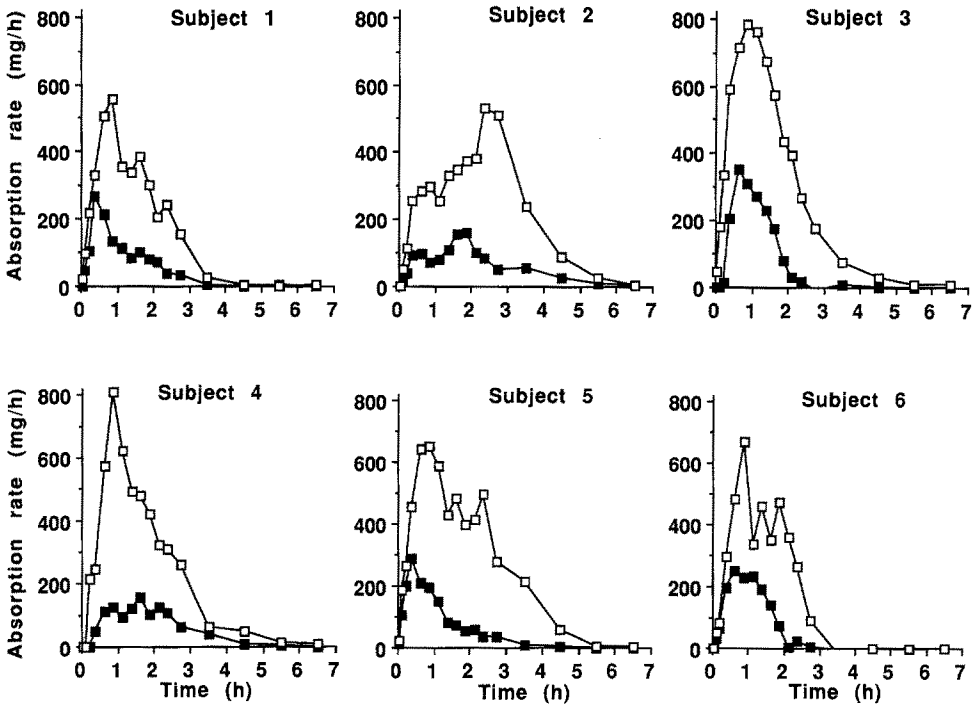

Fig. 2. Absorption rate of amoxicillin in 6 healthy subjects after the intake of 500 mg (■) and 3 g (□), using a deconvolution method

subjects. This confirms the previous observation of a "flip-flop" model for amoxicillin [20].

Third, absorption rate profiles were obtained using a deconvolution method (Fig. 2). These methods are especially useful when drug absorption is erratic and simple kinetic models do not apply [21]. However, deconvolution can give an incorrect estimate of the rate of absorption and negative input values [14, 22]. A few, small negative values were obtained here with the Iga method. After a short time lag, a peak in the absorption rate was observed in most of the subjects. However, contrary to the case of pure first-order absorption, the maximum absorption rate was not proportional to the dose and it was not followed by an exponential decline (Fig. 2). Absorption rate profiles could not be explained either by pure zero-order kinetics, as no saturation was displayed after the highest dose. Presentation of the input rate as the cumulative amount and fraction absorbed with time (Fig. 3) partly eases interpretation. Most of the curves include a straight portion, which denotes a zero-order process, and a trend towards a longer absorption time after the 3 g dose, as the curves are shifted to the right.

A decrease in the extent of the uptake of a drug absorbed by a simple first-order process cannot be explained by differences in absorption capacity in different parts of the intestine (the existence of a "window of absorption") if the transit time is not altered. A decrease in transit time with the dose is unlikely, as both doses were given with the same volume of water, and as a longer time of absorption was apparent with the higher oral dose in most of the subjects, by both the noncomparmental analysis and deconvolution. As the AUC has been shown to increase in proportion to the dose after IV doses up to 5 g [23], and renal clearance was found to be constant after oral doses up to 3 g [5], dose-dependent disposition of amoxicillin can be ruled out as an alternative explanation.

The solubility of amoxicillin reaches a minimum of 5.5 mg · ml^{-1} at pH 5 and at 37°C [2]. Incomplete solubility in combination with a limited gut segment where absorption could occur cannot be excluded as a contributing explanation for the decreased fraction absorbed after 3 g. However, solubility is unlikely to be the only explanation for the decrease in the fraction absorbed of a high dose [9, 24], and the existence of an absorption window is not compatible with the tendency to a longer absorption time observed with the higher dose. On the other hand, even if the lowest solubility is assumed, the 500 mg dose was taken with sufficient water to be totally dissolved (150 ml). Therefore, the apparent zero-order kinetics of absorption of the 500 mg dose, observed in some subjects both by compartment modelling and deconvolution, cannot be explained by limited solubility.

Saturable absorption kinetics in human have been reported for cefatrizine, another amino-β-lactam antibiotic, with good prediction of plasma concentrations by Michaelis-Menten type kinetics of absorption [15, 25]. The observation of partial linearity of the graphs of the cumulative amount of amoxicillin, reflecting a zero-order process, as in the report by Reigner et al. [15], is compatible with Michaelis-Menten absorption kinetics [26]. However, saturation was not observed after the high dose. This would mean that at least two uptake mechanisms operate for amoxicillin, as suggested by Sjövall et al. [5]. One is a specialised transport system and the other is diffusion. If so, the extent of absorption would approach a constant fraction of dose when the latter is excessive. Such a tendency was recently noted in a study of absorption in patients with an enterostomy given widely different doses of amoxicillin [9].

Westphal et al. [27] have observed a zero-order rate of absorption after 1 g amoxicillin, using the Loo-Riegelman method of analysis. The present results are not fully con-

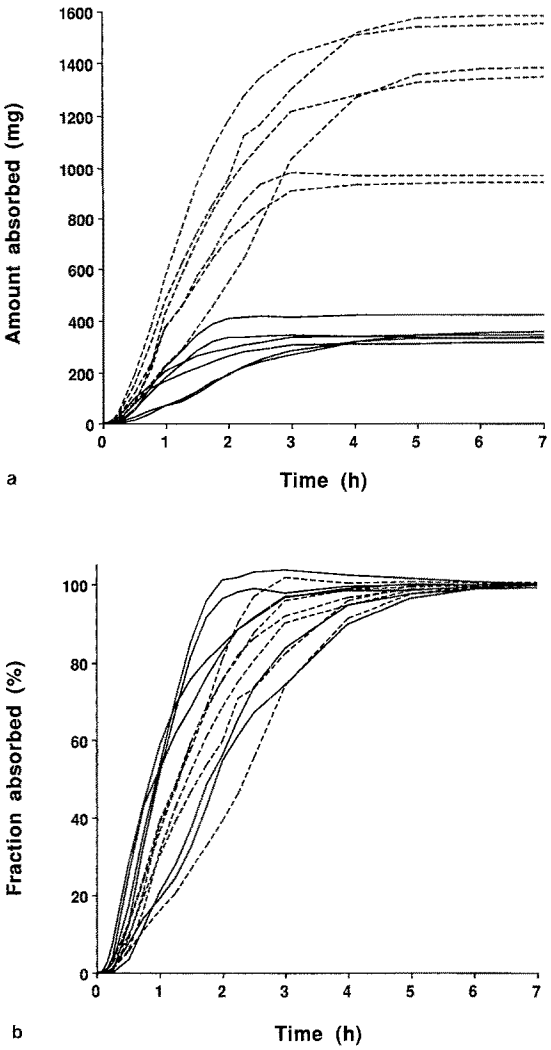

Fig. 3 a, b. Cumulative **a** amount and **b** fraction of amoxicillin absorbed with time in 6 healthy subjects after the intake of 500 mg (—) and 3 g (-----), calculated by a deconvolution method

sistent with theirs, as there was a more irregular profile of absorption here after both doses, and no saturation of the input rate after 3 g. The discrepancy may be due to the different drug formulations used; Westphal et al. gave amoxicillin as capsules and, as they pointed out, with a relatively small amount of water (100 ml) [27]. This may have led to disintegration or dissolution-limited absorption.

Although large inter-subject variability was observed and rate-limiting dissolution cannot be ruled out after the 3 g dose, the results are compatible with those reported from animal studies, where mixed Michaelis-Menten (predominant at low doses) and first-order kinetics of absorption (predominant at high doses) have been shown. However, specialised absorption of amoxicillin can be proved in man only by following the disappearance of dissolved drug from a closed gut reservoir or studying competition between drugs that share the same absorption system.

Acknowledgements. Supported by the Swedish MRC (3902) and the Funds of the Karolinska Institute. The fellowship of Dr. Paintaud was supported in part by grants from the Servier Research Group and LERS Synthelabo, France.

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