Determination of dose-dependent absorption of amoxycillin from urinary excretion data in healthy subjects

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Measurement of unchanged drug in urine was used to study the rate and extent of amoxycillin absorption after administration of amoxycillin sodium solution to six healthy subjects in a Latin-Square crossover design. The mean (95% CI) fraction of the dose excreted as unchanged amoxycillin decreased ($P < 0.05$) from 0.50 (0.44–0.56) after 97 mg amoxycillin sodium (= 0.25 mmol amoxycillin) to 0.23 (0.19–0.27) after 3103 mg (8 mmol), while the mean residence time determined from urinary excretion rate data increased ($P < 0.05$) from 1.54 (1.32–1.76) h to 2.16 (2.01–2.41) h. Plots of total urinary excretion and initial (0–30 min) excretion of unchanged drug vs dose indicated significant non-linearity above 776 mg doses. Michaelis-Menten parameters describing this relationship with respect to amount absorbed were 3.02 mmol for maximum amount absorbed and 1.93 mmol for amount absorbed at half maximum for 0–30 min. These results support a saturable absorption mechanism for amoxycillin which has clinical implications for high oral amoxycillin doses, and for competition with other drugs having capacity-limited absorption.

Keywords amoxycillin dose-dependent absorption Michaelis-Menten pharmacokinetics

Introduction

Despite its low lipophilicity and amphoteric nature amoxycillin is well absorbed when administered by mouth [1–3]. While little information is available on the mechanism(s) of absorption in man, non-linearity between bioavailability and dose [4–5] and demonstration of a zero-order absorption rate [3] has suggested that amoxycillin undergoes saturable absorption through the intestinal mucosa. In particular, amoxycillin may be partly or wholly absorbed by carrier-mediated transport processes similar to those responsible for the luminal absorption of di- and tri-peptides in rodents [6] and in man [7].

We show that either amoxycillin excretion over the first 30 min or total urinary recovery may be used to define non-linearity in the absorption of amoxycillin. The former method offers a more rapid and convenient means for investigating the mechanism(s) of amoxycillin absorption after administration to healthy subjects.

Methods

Six healthy subjects (three males, three females), aged 22–39 years, who were within normal limits for height and weight participated in the study. None had a history of penicillin allergy. Alcohol and smoking were prohibited from 24 h prior to, and during the study. No other medications including over-the-counter products were allowed for at least 1 week before or during the study. Strenuous physical activity was prohibited during the entire study period. The protocol was approved by the Human Research Ethics Committee of The University of Queensland, which is constituted according to the requirements of the National Health and Medical Research Council. All subjects gave written consent after the aims and procedures for the study were explained.

Amoxycillin sodium solution (Amoxil Parenteral; Beecham Research Laboratories, Dandenong, Vic., Australia) 97 mg, 194 mg, 388 mg, 776 mg, 1.552 g, and 3.103 g (equivalent to 0.25 mmol, 0.5 mmol,

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1 mmol, 2 mmol, 4 mmol and 8 mmol, respectively, of amoxycillin) was administered in an open, 6 × 6, Latin-Square crossover design on six different occasions. A washout period of at least 48 h separated the treatments. All subjects fasted for 12 h before each treatment. Tap water (150 ml) was given at 07.30 h and amoxycillin was administered at 08.00 h in 100 ml of sodium citrate buffer (0.01 M, pH 4.9) followed immediately by 150 ml of water. Further amounts of water (100 ml each) were given at 1, 2, 3 and 4 h after dosing, and *ad libitum* thereafter, to ensure adequate urine flow. The bladder was voided just before drug administration and at each urine collection time of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8 and 10 h after dosing. The urine volumes were recorded and 5 ml samples stored at −70°C until assayed.

Urinary concentrations of amoxycillin were measured by reversed-phase h.p.l.c. using solid-phase extraction [8]. Calibration plots were linear ($r > 0.999$), between-day and within-day imprecision was ≤ 15.5% (CV), and recovery was > 94%, for amoxycillin concentrations between 5–500 mg l^{-1}.

The elimination rate constant of amoxycillin (λ_z) was determined from the slope of the post-absorptive urinary excretion rate plot against time. The elimination half-life ($t_{1/2,z}$) was calculated from $0.693/\lambda_z$. The mean body residence time (MRT) of amoxycillin was calculated [9] from:

$$\text{MRT} = \frac{\sum_{i=1}^{i=n} [\frac{\Delta A_e}{\Delta t}]_i \cdot t_{mid}}{A_e (0, n)}$$

where,

$\dfrac{\Delta A_e}{\Delta t}$ = rate of excretion

t_{mid} = mid-point of urine collection

A_e = amount excreted

n = total number of urine collection periods

The relationship between amoxycillin dose (D) and the total amount absorbed (A) was described by a Michaelis-Menten type of equation:

$$A = \frac{A_{max} \cdot D}{(K_d + D) \cdot f_e}$$

where, A_{max} is the theoretical maximum and f_e is the fraction of an i.v. dose which is excreted unchanged; a constant value of 0.55 was assumed for the latter [10].

The parameters A_{max} and K_d were estimated by nonlinear regression analysis using the Simplex algorithm [11] in PCNONLIN (v.4, SCI Software, Lexington, KY, U.S.A.). Initial graphical estimates of these parameters were varied by ± 25% and the data were reanalysed to ensure unique convergence. The pharmacokinetic data were analysed statistically using 3-way ANOVA with subjects, period, and treatments as the main effects.

Results

The cumulative excretion of unchanged amoxycillin after different doses reached an asymptote after about 6 h, and mean maximum excretion rates of drug occurred between 0.5 and 1.5 h after administration, indicating its rapid absorption. The total amount excreted and the initial rate of excretion increased less than proportionately with increasing dose above 2 mmol (Table 1). There was no significant change in the elimination half-life, but mean residence time increased by about forty percent ($P < 0.05$) when amoxycillin dose was increased from 0.25 mmol to 8 mmol (Table 1). Less than one-quarter of the dose was excreted following administration of 8 mmol compared with one-half from a 0.25 mmol dose. The period (order of administration) effect was insignificant ($P > 0.05$). Mean (95% confidence limits) values of the apparent (0–30 min data) Michaelis-Menten constants were 0.36 (0.17–0.54) mmol h^{-1} for A_{max} and 3.52 (0.31–6.73) mmol for K_d. Furthermore, there was a strong linear association ($r = 0.996$)

Table 1 Pharmacokinetics of amoxycillin evaluated from urinary excretion data following administration of amoxycillin sodium solution to 6 normal subjects. Values are expressed as mean (95% CI)

Dose[1]			MRT	$t_{1/2,z}$
mg	mmol[2]	f_e	(h)	(h)
97	0.25	0.50 (0.44–0.56)	1.54 (1.32–1.76)	1.03 (0.80–1.23)
194	0.50	0.50 (0.46–0.54)	1.67 (1.45–1.89)	1.13 (1.03–1.23)
388	1.00	0.50 (0.47–0.53)	1.77 (1.52–2.02)	1.13 (1.01–1.25)
776	2.00	0.45 (0.41–0.49)	1.89 (1.62–2.16)	1.10 (1.00–1.20)
1552	4.00	0.37 (0.32–0.42)	2.15 (1.90–2.40)	1.24 (1.12–1.36)
3103	8.00	0.23 (0.19–0.27)	2.16 (2.01–2.41)	1.26 (1.12–1.40)

[1]Dose of amoxycillin sodium (mg); [2]Amoxycillin equivalents.

between total amount excreted and excretion over the first 30 min across doses (Figure 1).

Discussion

Depending on dose and route more than half of an amoxycillin dose is excreted renally. Thus, measurement of urinary recovery of the drug offers a convenient and non-invasive means of estimating amoxycillin absorption [2]. Amoxycillin sodium solution was used since commercially available suspensions and capsules contain amoxycillin trihydrate, which has an aqueous solubility of only 5.5 mg ml^{-1} at 37°C and pH 5.5 [12]. Since these conditions are present in the luminal fluids of the small intestine there is the possibility that valid interpretation of absorptive processes may be confounded by limited solubility of the trihydrate at higher doses [13]. Our preliminary experiments indicated that solutions of the lowest (0.25 mmol) and highest (8 mmol) doses of amoxycillin sodium produced no precipitation of amoxycillin between pH 2.0–7.5.
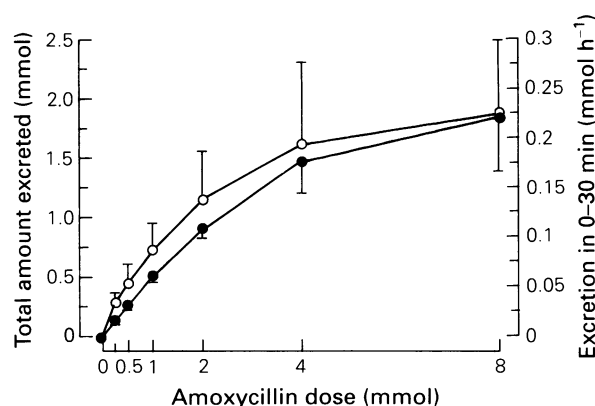
Use of data collected over the first 30 min instead of the minimum 6 h for total amount, potentially provides a more sensitive means of studying influences on the absorption rate of amoxycillin. For example, our preliminary (unpublished) results showed that the initial rate of excretion, but not total amount of drug excreted, was decreased significantly when amoxycillin was taken with a 100-fold excess of either glycylproline, glycylleucine, glycyltyrosine, or glycylglycine.

Although the analysis of urinary excretion data is an indirect means of studying drug absorption, the present results are consistent with saturable absorption above 2 mmol doses of amoxycillin. The method assumes that clearance and volume of distribution of drug are not dose-dependent. The mean terminal half-life of amoxycillin was similar after the six doses. Thus, any change in clearance would need to be balanced by a commensurate change in volume (and vice versa), an unlikely occurrence. Furthermore, others have reported that amoxycillin kinetics are linear over a wide dose-range after intravenous administration [14]. It is also unlikely that our data reflected alterations in gastric emptying and intestinal motility since all treatments were uniform with respect to volume, strength and pH of the buffer solution containing amoxycillin sodium, and the volume of water administered before and after drug administration. There is no evidence that stomach emptying or intestinal motility is affected by the dose of amoxycillin. Finally, it is recognised that the renal clearance of some drugs can be influenced by urine flow, particularly those which are extensively reabsorbed [15, 16]. Although fluid intake was controlled in the present study, analyses of variance confirmed that urine flow differed insignificantly ($P > 0.05$) among doses, and with the order in which amoxycillin was administered.

The method we have described for assessing the absorption of amoxycillin is presently being used by us to determine the role of the putative small peptide carrier [7] in the absorption of this antibiotic from the intestinal tract in man.

Figure 1 Relationships between mean (± s.d.) total urinary recovery (●) and 0–30 min recovery (○) of unchanged amoxycillin and dose of amoxycillin sodium (in amoxycillin equivalents).

References

1 Welling PG, Huang H, Koch PA, Craig WA, Madsen PO. Bioavailability of ampicillin and amoxicillin in fasted and nonfasted subjects. *J pharm Sci* 1977; **66**: 549–552.

2 Arancibia A, Guttmann J, González G, González C. Absorption and disposition kinetics of amoxicillin in normal human subjects. *Antimicrob Agents Chemother* 1980; **17**: 199–202.

3 Hespe W, Verschoor JSC, Olthoff M. Bioavailability of new formulations of amoxicillin in relation to its absorption kinetics. *Arzneim-Forsch/Drug Res* 1987; **37**: 372–375.

4 Sjövall J, Alván G, Westerlund D. Dose-dependent absorption of amoxycillin and bacampicillin. *Clin Pharmac Ther* 1985; **38**: 241–250.

5 Arancibia A, Icarte A, González C, Morasso I. Dose-dependent bioavailability of amoxycillin. *Int J clin Pharmac Ther Toxicol* 1988; **26**: 300–303.

6 Tsuji A, Nakashima E, Kagami I, Yamana T. Intestinal absorption mechanism of amphoteric β-lactam antibiotics I: Comparative absorption and evidence for saturable transport of amino-β-lactam antibiotics by *in situ* rat small intestine. *J pharm Sci* 1981; **70**: 768–772.

7 Westphal JF, Deslandes A, Brogard JM, Carbon C.

Reappraisal of amoxycillin absorption kinetics. *J Antimicrob Chemother* 1991; **27**: 647–654.

8 Chulavatnatol S, Charles BG. High-performance liquid chromatographic determination of amoxycillin in urine using solid-phase, ion-pair extraction and ultraviolet detection. *J Chromatogr* 1993; **615**: 91–96.

9 Delgado Charro MB, Vila Jata JL. In vivo study of sustained-release formulations containing amoxicillin and Gelucire 64/02. *Int J Pharm* 1992; **78**: 35–41.

10 Hill SA, Jones KH, Lees LJ. Pharmacokinetics of parenterally administered amoxycillin. *J Infect* 1980; **2**: 320–332.

11 Nelder JA, Mead R. A simplex method for function minimisation. *Comput J* 1965; **7**: 308–313.

12 Tsuji A, Nakashima E, Hamano S, Yamana T. Physicochemical properties of amphoteric β-lactam antibiotics I: stability, solubility and dissolution behaviour of amino penicillins as a function of pH. *J pharm Sci* 1978; **67**: 1059–1066.

13 Torres-Molina F, Peris-Ribera JE, García-Carbonell MC, Aristorena JA, Plá-Delfina JM. Nonlinearities in amoxycillin pharmacokinetics II. Absorption studies in the rat. *Biopharm Drug Dispos* 1992; **13**: 39–53.

14 Paintaud G, Alván G, Dahl ML, Grahnén A, Sjövall J, Svensson JO. Nonlinearity of amoxicillin absorption kinetics in humans. *Eur J clin Pharmac* 1992; **43**: 283–288.

15 Birkett DJ, Miners JO. Caffeine renal clearance and urine caffeine concentrations during steady state dosing. Implications for monitoring caffeine intake during sports events. *Br J clin Pharmac* 1991; **31**: 405–408.

16 Gibaldi M. Drug disposition – Elimination. In *Biopharmaceutics and clinical pharmacokinetics*, Fourth Edition, Philadelphia: Lee & Febiger, 1991: 203–233.