Br. vet. J. (1996). **152**, 551

PHARMACOKINETICS OF AMOXICILLIN/CLAVULANIC ACID COMBINATION AND OF BOTH DRUGS ALONE AFTER INTRAVENOUS ADMINISTRATION TO GOATS

E. ESCUDERO*, C. M. CARCELES and S. VICENTE

Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, University of Murcia, Campus de Espinardo, 30.071-Murcia, Spain

SUMMARY

The pharmacokinetic behaviour of an amoxicillin/clavulanic acid combination (25 mg kg^{-1}), and both drugs alone (amoxicillin 20 mg kg^{-1}), clavulanic acid 5 mg kg^{-1}), was studied after intravenous (i.v.) administration of single doses to 10 goats. The objective was to determine whether there were differences in the plasma kinetics of these drugs when administered in combination or alone. The plasma concentration–time data were analysed by compartmental pharmacokinetics and non-compartmental methods. The disposition curves for both drugs alone and in combination were best described by a biexponential equation (two-compartment open model). The elimination half-lives of amoxicillin were 1.05±0.09 h alone and 1.13±0.19 h in combination, and those of clavulanic acid were 0.87± 0.07 h and 0.85±0.09 h, respectively. The apparent volumes of distribution of amoxicillin and clavulanic acid were similar in the two treatments. Body clearances of amoxicillin were 0.12±0.01 l h^{-1} · kg alone and 0.11± 0.01 l h^{-1} · kg in combination, and of clavulanic acid were 0.12± 0.02 l h^{-1} · kg alone and 0.12±0.01 l h^{-1} · kg in combination with amoxicillin. The half-lives and body clearances of amoxicillin and clavulanic acid did not differ significantly when administered alone and in combination. It was concluded that the i.v. administration of amoxicillin and clavulanic acid as a combination product did not alter the disposition kinetics of either drug.

KEYWORDS: Pharmacokinetics; amoxicillin; clavulanate; goats; intravenous.

INTRODUCTION

Amoxicillin is a broad spectrum antibiotic of the penam penicillin group, useful for treating urinary tract, respiratory and skin bacterial infections in animals. In

*To whom correspondence should be addressed.

0007-1935/96/050551-09/$12.00/0

dogs, the systemic availability of amoxicillin (60–70%) is about twice that of ampicillin (20–40%), so that plasma amoxicillin concentrations are often twice or more than those that occur after the same oral dose of ampicillin (Prescott & Baggot, 1993). However, this antibiotic has the disadvantage of inactivation by bacterial β-lactamases or penicillinases (Sykes & Matthew, 1976; Rolinson, 1986).

The addition of a penicillinase inhibitor, such as clavulanic acid, results in a drug combination that broadens the antibacterial spectrum of amoxicillin. Clavulanic acid is a natural substance produced by *Streptomyces clavuligerus*, and has slight antibacterial activity but a very high affinity for many β-lactamases. This compound is structurally similar to penicillins and reacts with β-lactamases resulting in a progressive inactivation of the enzyme (Brodgen *et al.*, 1981; Rolinson, 1986). The inactivation of β-lactamases by clavulanate protects susceptible β-lactam antibiotics, such as penicillin G, ampicillin, amoxicillin, ticarcillin, carbenicillin and certain cephalosporins from hydrolytic destruction, maintaining their antimicrobial activity against many β-lactamase-producing bacteria (Reading & Cole, 1977; Wise *et al.*, 1978; Farrel & Brookes, 1980; Hunter *et al.*, 1980). Amoxicillin is combined with potassium clavulanate in a 4:1 ratio. The combination is not destroyed by gastric or intestinal secretions and is rapidly absorbed. Combinations of amoxicillin and clavulanic acid showed increased effectiveness against an experimental model of acute bacterial cystitis in cats infected with *Escherichia coli* with *in vitro* resistance to amoxicillin (Senior *et al.*, 1985) and in the treatment of skin infections under both experimental and clinical conditions in dogs (Bywater *et al.*, 1985). The pharmacokinetic behaviour of this combination has been studied in man (Weber *et al.*, 1986) and in several animal species (Mizen *et al.*, 1981; Cooper, 1985). However, no references have been found on the disposition kinetics of amoxicillin/clavulanic acid in goats. The objective of the present study was to compare the pharmacokinetics of amoxicillin and clavulanic acid in goats after intravenous (i.v.) administration of the amoxicillin/clavulanic acid combination and each drug alone in order to determine whether the presence of clavulanic acid alters the disposition kinetics of amoxicillin.

MATERIALS AND METHODS

Animals

Ten Murciano–Granadina goats weighing between 38 and 43 kg were used as experimental animals. All animals were female, milk-producing (in lactation phase), and in good health. The animals were stabled and fed an antibiotic-free diet for at least 30 days preceding the study. Water was available *ad libitum*.

Study design

A crossover study was conducted in three phases separated by 30 days. In the first phase, the combination, an aqueous solution of amoxicillin:clavulanic acid (4:1 ratio), was administered i.v. to the animals at a dosage of 25 mg kg^{-1} body weight (20 mg kg^{-1} of sodium amoxicillin and 5 mg kg^{-1} of potassium clavulanate; generously supplied by Antibioticos, S.A. Laboratories). In the second and third phases, aqueous solutions of amoxicillin (20 mg kg^{-1}) or clavulanic acid

(5 mg kg^{-1}), respectively, were injected into the left jugular vein. All animals were fasted 24 h before each injection. Blood samples were collected from the contralateral jugular vein into heparinized 'Vacutainer' tubes at 0 (pre-treatment), 0.05, 0.10, 0.15, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 7 and 9 h after drug administration. The samples (5 ml) were centrifuged at 1500 *g* for 15 min and the plasma was separated and stored for one day at −40°C until assayed.

Analytical method

Plasma concentrations of amoxicillin and clavulanic acid were analysed by high performance liquid chromatography (HPLC) using a Waters HPLC unit equipped with a 721 model integrator, a Lambda-Max LS 481 model detector and two 510 model pumps, according to the method of Foulstone & Reading (1982). The column used was a μBondapack C_{18} (Waters) with a guard-pack precolumn with the same filling. The mobile phase was 6% methanol plus phosphate buffer with pH adjusted to 3.2 and the flow rate was 2 ml min^{-1}. The concentrations were scanned by a UV detector at 227 nm for amoxicillin and 311 nm for clavulanic acid, and 0.005 absorbance units full scale (AUFS). In the clavulanic acid assay, the plasma samples were derivatized (Foulstone & Reading, 1982) using imidazole reagent (8.25 g imidazole in 24 ml distilled water with the pH adjusted to 6.8 by the addition of 5 M HCl and the volume made up to 40 ml with distilled water). The sample (0.4 ml) to be assayed was added to the imidazole reagent (0.1 ml) and, after a 10 min reaction period at room temperature, 25 μl were injected onto the HPLC column. The limits of detection were 0.45 μg/ml for amoxicillin and 0.09 μg ml^{-1} for clavulanic acid.

Pharmacokinetics

The concentration–time data for amoxicillin and clavulanic acid were initially analysed by the method of residuals (Gibaldi & Perrier, 1982) and PKCALC computer programme (Shumaker, 1986) to obtain the best estimates of the parameters. Refinement of the estimates was carried out using the MULTI computer programme and Gauss–Newton damping algorithm (Yamaoka *et al.*, 1981). Wagner's criterion (1983) was used to calculate the weighting coefficients ($1/C_p^W$) according to the expression:

$$2 \cdot \log (\text{SD}) = \log a + W \cdot \log C$$

where SD is the standard deviation, a is the intercept with the ordinate, W is the slope of the linear regression (weighting coefficient) and C is mean concentration in plasma.

The pharmacokinetic parameters were calculated from the final equations obtained (Gibaldi & Perrier, 1982) and the area under the plasma concentration–time curve (AUC) was calculated using the trapezoidal logarithmic method with extrapolation to infinite time. The symbols, equations and definitions used in this pharmacokinetic study were standard (Aronson *et al.*, 1988).

Statistical analysis

The usual statistical parameters were calculated and the Kolmogorov–Smirnov

test was employed to verify the homogeneity of the data and to test for between-animal differences in the parameters. The Mann–Whitney test and Student's *t* test were used to test parameters for significant differences when amoxicillin and clavulanic acid were administered alone or in combination to goats (Powers, 1990).

RESULTS

The pharmacokinetics of both amoxicillin and clavulanic acid given i.v. and separately could best be described by the following biexponential equations:

$$C=192.09 \cdot e^{-11.826t}+96.46 \cdot e^{-0.654t} \text{ (amoxicillin)}$$

$$C=61.51 \cdot e^{-8.718t}+28.55 \cdot e^{-0.7981t} \text{ (clavulanic acid)}$$

The biexponential equations that best described the kinetics of amoxicillin and clavulanic acid given in combination were:

$$C=149.31 \cdot e^{-9.2382t}+102.38 \cdot e^{-0.6194t} \text{ (amoxicillin)}$$

$$C=67.79 \cdot e^{-10.0635t}+29.12 \cdot e^{-0.8238t} \text{ (clavulanic acid)}$$

where concentrations are expressed in mg l^{-1} and time in h. The mean (±SD) plasma concentrations following the administration of both antibiotics given alone are plotted in Fig. 1. The mean (±SD) plasma concentration–time data for the antibiotics given in combination are plotted in Fig. 2.

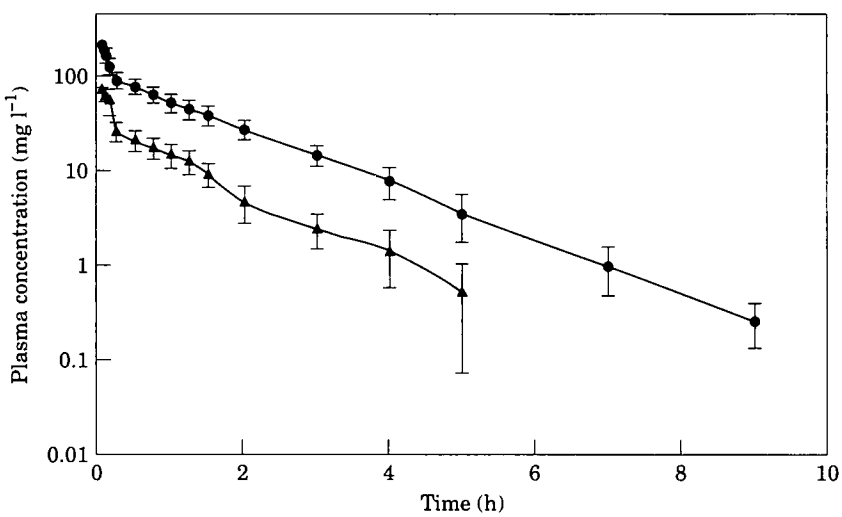

Fig. 1. Plasma concentrations (mean±SD) of amoxicillin and clavulanic acid in goats following intravenous administration at a dosage of 20 mg kg^{-1} of amoxicillin and 5 mg kg^{-1} of clavulanic acid. (●), Amoxicillin; (▲), clavulanic acid.

The pharmacokinetic parameters (mean±SD) for each antibiotic and manner of administration (alone or in combination) based on compartmental pharmacoki-

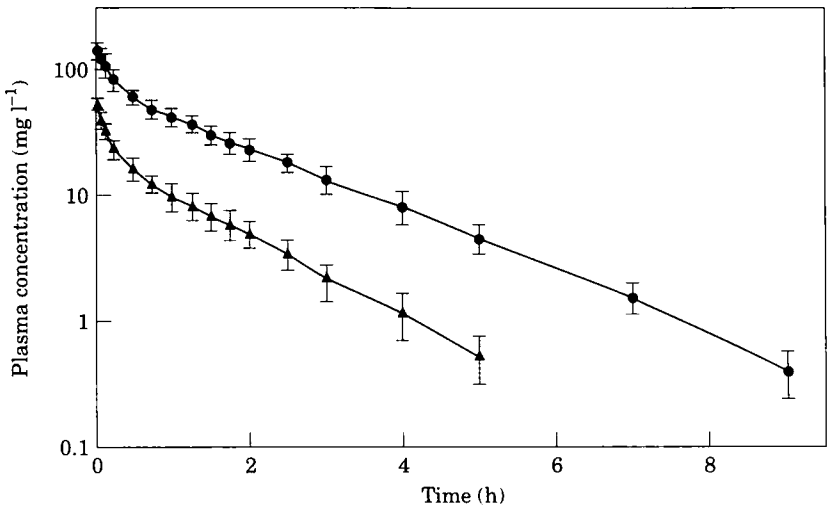

Fig. 2. Plasma concentrations (mean±SD) of amoxicillin/clavulanic acid combination in goats following intravenous administration at a dosage of 25 mg kg^{-1} (20 mg kg^{-1} of amoxicillin and 5 mg kg^{-1} of clavulanic acid). (●), Amoxicillin; (▲), clavulanic acid.

Table I
Pharmacokinetic parameters (mean±SD) of amoxicillin in goats given intravenously alone and as amoxicillin/clavulanic acid combination at a dosage of 20 mg kg^{-1}

Parameters		*Alone*	*Combination*
C_1	(mg l^{-1})	192.09±6.22	149.31±23.25
C_2	(mg l^{-1})	96.46±5.51	102.38±13.28
λ_1	(h^{-1})	11.83±1.41	9.24±5.35
λ_2	(h^{-1})	0.65±0.06	0.62±0.10
$t_{1/2\lambda 1}$	(h)	0.06±0.02	0.09±0.04
$t_{1/2\lambda 2}$	(h)	1.07±0.09	1.12±0.19
V_{area}	(l kg^{-1})	0.19±0.02	0.18±0.02
V_{ss}	(l kg^{-1})	0.16±0.02	0.16±0.02
AUC	(mg · h l^{-1})	163.18±22.15	186.17±21.33
MRT	(h)	1.47±0.19	1.52±0.20
CL	(l h^{-1} · kg)	0.12±0.01	0.11±0.01

C_1: Intercept of the ordinate by the fastest disposition slope minus the intercept of the next fastest disposition slope. C_2: Intercept of the slowest disposition slope with the ordinate. λ_1: Distribution rate constant. λ_2: Elimination rate constant. $t_{1/2\lambda 1}$: The distribution half-life associated with the initial slope (λ_1) of a semilogarithmic concentration–time curve. $t_{1/2\lambda 2}$: The apparent elimination half-life associated with the terminal slope (λ_2) of a semilogarithmic concentration–time curve. V_{area}: The apparent volume of distribution calculated by the area method. V_{ss}: The apparent volume of distribution at steady state. AUC: The area under the plasma concentration–time curve from zero to infinity. CL: The total body clearance of drug from the plasma. MRT: Mean residence time.

Table II
Pharmacokinetic parameters (mean±SD) of clavulanic acid in goats given intravenously alone and as amoxicillin/clavulanic acid combination at a dosage of 5 mg kg^{-1}

Parameters		*Alone*	*Combination*
C_1	(mg l^{-1})	61.51±3.71	67.79±6.12
C_2	(mg l^{-1})	28.55±2.93	29.11±3.21
λ_1	(h^{-1})	8.72±1.23	10.06±1.68
λ_2	(h^{-1})	0.80±0.04	0.82±0.09
$t_{1/2\lambda 1}$	(h)	0.08±0.01	0.07±0.01
$t_{1/2\lambda 2}$	(h)	0.87±0.07	0.85±0.09
V_{area}	(l kg^{-1})	0.15±0.01	0.14±0.01
V_{ss}	(l kg^{-1})	0.13±0.02	0.12±0.01
AUC	(mg · h l^{-1})	42.93±5.10	42.60±5.31
MRT	(h)	1.01±0.02	1.04±0.13
CL	(l h^{-1} · kg)	0.12±0.01	0.12±0.01

C_1: Intercept of the ordinate by the fastest disposition slope minus the intercept of the next fastest disposition slope. C_2: Intercept of the slowest disposition slope with the ordinate. λ_1: Distribution rate constant. λ_2: Elimination rate constant. $t_{1/2\lambda 1}$: The distribution half-life associated with the initial slope (λ_1) of a semilogarithmic concentration–time curve. $t_{1/2\lambda 2}$: The apparent elimination half-life associated with the terminal slope (λ_2) of a semilogarithmic concentration–time curve. V_{area}: The apparent volume of distribution calculated by the area method. V_{ss}: The apparent volume of distribution at steady state. AUC: The area under the plasma concentration–time curve from zero to infinity. CL: The total body clearance of drug from the plasma. MRT: Mean residence time.

netic analysis and non-compartmental methods are presented in Tables I and II. There were significant differences between each treatment in some of the pharmacokinetic parameters. For amoxicillin, the significant differences ($P<0.05$) were in the zero-time intercept of the ordinate by the initial disposition slope (C_1), and the non-compartmental parameter area under the concentration–time curve (AUC). For clavulanic acid, significant differences ($P<0.05$) were found in the initial disposition rate constant (λ_1) and the zero-time intercept of the initial disposition phase (C_1).

DISCUSSION

Using Akaike's information criterion (Yamaoka *et al.*, 1978), it was found that the amoxicillin and clavulanic acid plasma concentration *vs* time data after i.v. administration of both drugs alone and in combination were best fitted to a two-compartment open model in goats. This conclusion is in agreement with that found in previous studies carried out in various animal species (Mizen *et al.*, 1981; Bolton *et al.*, 1984; Senior *et al.*, 1985) and in man (Brodgen *et al.*, 1981; Weber *et al.*, 1986).

For both antibiotics, no significant differences between the drugs alone and in combination were found in the apparent volume of distribution at steady-state (V_{ss}) and the apparent volume of distribution (V_{area}) calculated by the area method. In other species it had been found that V_{area} values for amoxicillin were greater than in the present study (0.19 l kg^{-1}): 0.49 l kg^{-1} in horses (Montesissa *et*

al., 1988), 1.51 l kg^{-1} in pigeons (Dorrestein *et al.*, 1987), 0.95 l kg^{-1} in goats and 0.67 l kg^{-1} in sheep (Craigmill *et al.*, 1992). The V_{area} and V_{ss} values (0.21 and 0.16 l kg^{-1}, respectively) found for clavulanic acid combined with ticarcillin in 28-day-old foals (Wilson *et al.*, 1991) were close to the data presented here (0.15 and 0.13 l kg^{-1} respectively).

Significant differences ($P<0.05$) were found in amoxicillin and clavulanic acid half-lives ($t_{1/2\lambda 2}$) within each treatment; amoxicillin had a longer half-life, 1.07 h, than clavulanic acid, 0.87 h. A shorter half-life for amoxicillin than in the present study was reported previously in lactating dairy cows (0.75 h) after i.v. administration of 10 mg kg^{-1} (Ziv & Nouws, 1979) and in sheep (0.77 h), but a longer half-life was reported in goats (1.11 h) (Craigmill *et al.*, 1992).

The values for the kinetic terms describing the disposition of clavulanate indicated that the drug had a significantly more rapid, but limited, distribution than amoxicillin and had a slight (but not significant) effect on the half-life of amoxicillin when the combination was used. The half-lives reported in 3- and 28-day-old foals of 0.73 and 0.45 h, respectively, for clavulanic acid combined with ticarcillin (Wilson *et al.*, 1991) were shorter than those found in the present study for goats. In humans, the half-life of clavulanic acid was 1.3 h after i.v. infusion at a dosage of 1.87 mg kg^{-1} body weight (Hampel *et al.*, 1988). Mean residence time (MRT), which is the statistical moment analogy to half-life, was similar for each drug whether administered alone or in combination.

The values of body clearances obtained for amoxicillin, 0.12 l $h^{-1} \cdot kg$, were lower than those reported by Montesissa *et al.* (1988) in horses (239 ml $h^{-1} \cdot kg$), Khanikor *et al.* (1986) in buffalo calves (0.342 l $h^{-1} \cdot kg$), Craigmill *et al.* (1992) in goats (0.606 l $h^{-1} \cdot kg$) and sheep (0.684 l $h^{-1} \cdot kg$) and Rutgers *et al.* (1980) in dairy cows (0.36 l $h^{-1} \cdot kg$). For clavulanic acid, Wilson *et al.* (1991) reported a higher value (0.313 l $h^{-1} \cdot kg$) of body clearance in foals than was found in goats (0.12 l $h^{-1} \cdot kg$).

The present results indicate that the pharmacokinetics of an amoxicillin/clavulanic acid combination and both drugs alone injected i.v. in goats, did not differ significantly. In both treatments the half-life of clavulanic acid were significantly shorter than that of amoxicillin, but clearances values (Cl) of the drugs were not significantly different.

In conclusion, clavulanic acid administered as amoxicillin:clavulanate combination did not change the disposition kinetics of amoxicillin in goats, but broadens its antibacterial spectrum of action (Brodgen *et al.*, 1981).

REFERENCES

ARONSON, J. K., DENGLER, H. J., DETTLI, L. & FOLLATH, F. (1988). Standardization of symbols in clinical pharmacology. *European Journal of Clinical Pharmacology* **35,** 1–7.

BOLTON, G. C., ALLEN, G. D., FILER, C. W. & JEFFERY, D. J. (1984). Absorption, metabolism and excretion studies on clavulanic acid in the rat and the dog. *Xenobiotica* **14,** 483–90.

BRODGEN, R. N., CARMINE, A., HEEL, R. C., MARLEY, P. A., SPEIGHT, T. M. & AVERY, G. S. (1981). Amoxicillin/clavulanic acid: a review of its antibacterial activity. Pharmacokinetics and therapeutic use. *Drugs* **22,** 337–62.

BYWATER, R. J., PALMER, G. H., BUSWELL, J. F. & STANTON, A. (1985). Clavulanate-potentiated amoxycillin: activity *in vitro* and bioavailability in the dog. *The Veterinary Record* **12,** 33–6.

COOPER, J. E. (1985). Safety and efficacy of clavulanate-potentiated amoxycillin in pigeons (*Columba livia*). *Research in Veterinary Science* **39,** 87–9.

CRAIGMILL, A. L., PASS, M. A. & WETZLICH, S. (1992). Comparative pharmacokinetics of amoxicillin administered intravenously to sheep and goats. *Journal of Veterinary Pharmacology and Therapeutics* **15,** 72–7.

CORRESTEIN, G. M., VAN GOGH, H., RINZEMA, J. D. & BUITELAAR, M. N. (1987). Comparative study of ampicillin and amoxycillin after intravenous, intramuscular and oral administration in homing pigeons (*Columba livia*). *Research in Veterinary Science* **42,** 343–8.

FARREL, I. D. & BROOKES, G. R. (1980). The laboratory efficacy of amoxicillin and clavulanic acid combination. In *Augmentin: clavulanate-potentiated amoxycillin.* eds Rolinson, G. N. & Watson, A. pp. 54–7. Amsterdam: Excerpta Medica.

FOULSTONE, M. & READING, C. (1982). Assay of amoxycillin and clavulanic acid, the components of Augmentin, in biological fluids with high-performance liquid chromatography. *Antimicrobial Agents and Chemotherapy* **22,** 753–62.

GIBALDI, M. & PERRIER, D. (1982). *Pharmacokinetics,* 2nd Edn, p. 433. New York: Marcel Dekker.

HAMPEL, B., LODE, H., BRUCKNER, G. & KOEPPE, P. (1988). Comparative pharmacokinetics of sulbactam/ampicillin and clavulanic acid/amoxycillin in human volunteers. *Drugs* **35,** (Suppl. 7), 29–33.

HUNTER, P. A., COLEMAN, K., FISHER, J. & TAYLOR, D. (1980). *In vitro* synergistic properties of clavulanic acid with ampicillin, amoxycillin and ticarcillin. *Journal of Antimicrobial Chemotherapy* **6,** 445–70.

KHANIKOR, H. N., SRIVASTAVA, A. K., PAUL, B. S. & MALIK, J. K. (1986). Kinetic disposition and biodistribution of amoxycillin in *Bubalis bubalis. Zentralblatt für Veterinärimedizin A,* **33,** 212–8.

MIZEN, L., BHANDAIR, K., SAYER, J. & CATHERRALL, E. (1981). Pharmacokinetics and distribution of Augmentin (amoxycillin and clavulanic acid) in laboratory animals. *Drugs under Experimental and Clinical Research* **7,** 263–7.

MONTESISSA, C., CARLI, S., SONZOGNI, O., GARLAPPI, R. & SERICOLA, A. (1988). Pharmacokinetics of sodium amoxycillin in horses. *Research in Veterinary Science* **44,** 233–6.

POWERS, J. (1990). Statistical analysis of pharmacokinetic data. *Journal of Veterinary Pharmacology and Therapeutics* **13,** 113–20.

PRESCOTT, J. F. & BAGGOTT, J. D. (1993). Beta-lactam antibiotics: penam penicillins. In *Antimicrobial Therapy in Veterinary Medicine.* eds Prescott, J. F. & Baggott, J. D., 2nd Edn., p 92. Ames: Iowa State University Press.

READING, C. & COLE, M. (1977). Clavulanic acid: a β-lactamase-inhibiting β-lactam from *Streptomyces clavuligerus. Antimicrobial Agents and Chemotherapy* **11,** 852–7.

ROLINSON, G. N. (1986). Inhibidores de las beta-lactamasas. *Revista Española de Microbiología Clínica* **1,** 13–20.

RUTGERS, L. J. E., VAN MIERT, A. S. J. P. A. M., NOUWS, J. F. M. & VAN GINNEKEN, C. A. M. (1980). Effect on the injection site on the bioavailability of amoxicillin trihydrate in dairy cows. *Journal of Veterinary Pharmacology and Therapeutics* **3,** 125–32.

SENIOR, D. F., GASKIN, J. M., BUERGELT, C. D., FRANKS, P. P. & KEEFE, T. J. (1985). Amoxycillin and clavulanic acid combination in the treatment of experimentally induced bacterial cystitis in cats. *Research in Veterinary Science* **39,** 42–6.

SHUMAKER, R. C. (1986). PKCALC: a basic interactive computer program for statistical and pharmacokinetic analysis of data. *Drug Metabolism Reviews* **17,** 331–48.

SYKES, R. B. & MATTHEW, M. (1976). The beta-lactamases of Gram-negative bacteria and their role in resistance to beta lactam antibiotics. *Journal of Antimicrobial Chemotherapy* **2,** 115–57.

WAGNER, J. G. (1983). *Farmacocinética clínica.* Editorial Reverté, S. A. Barcelona pp. 295–6.

WEBER, D. J., TOLKOFF-RUBIN, N. E. & RUBIN, R. H. (1986). Amoxicilina y clavulanato potásico: una combinación antibiótica. Mecanismo de acción, farmacocinética, espectro antimicrobiano, eficacia clínica y efectos adversos. *Revista Española de Microbiología Clínica* **1,** 214–29.

WILSON, W. D., SPENSLEY, M. S., BAGGOTT, J. D., HIETALA, S. K. & PRIOR, P. (1991). Pharmacokinetics and bioavailability of ticarcillin and clavulanate in foals after intravenous and intramuscular administration. *Journal of Veterinary Pharmacology and Therapeutics* **14,** 78–89.

WISE, R., ANDREWS, J. M. & BEDFORD, K. A. (1978). *In vitro* study of clavulanic acid in combination with penicillin, amoxycillin and carbenicillin. *Antimicrobial Agents and Chemotherapy* **13,** 389–93.

YAMAOKA, K., NAKAGAWA, T. & UNO, T. (1978). Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equations. *Journal of Pharmacokinetics and Biopharmaceutics* **6,** 165–75.

YAMAOKA, K., TAMIGAWARA, Y., NAKAGAWA, T. & UNO, T. (1981). A pharmacokinetic analysis program (MULTI) for microcomputer. *Journal of Pharmacobio-Dynamics* **4,** 879–85.

ZIV, G. & NOUWS, J. F. M. (1979). Serum and milk concentrations of ampicillin and amoxycillin in ruminants. *Refuah Veterinarith* **36** (3), 104–10.

(Accepted for publication 26 April 1995)