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The Disposition Kinetics, Urinary Excretion and Dosage Regimen of Ciprofloxacin in Buffalo Calves (*Bubalus bubalis*)

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ABSTRACT

The disposition kinetics, urinary excretion and a dosage regimen for ciprofloxacin after a single intravenous administration of 5 mg/kg was investigated in 5 healthy buffalo calves. The disposition kinetics were best fitted to a three-compartment open model. After 1 min, the concentration of ciprofloxacin in plasma was 8.50 ± 0.39 μg/ml and the minimum therapeutic concentration was maintained for 10 h. The elimination half-life and volume of distribution were 3.88 and 0.08 h and 3.97 ± 0.22 L/kg, respectively. The total body clearance and T/P ratio were 0.709 ± 0.025 L/kg per h and 6.13 ± 0.54, respectively. Approximately 28.3% of the total administered dose of ciprofloxacin was recovered in urine within 24 h of administration. To maintain a minimum therapeutic plasma concentration of 0.10 μg/ml, a satisfactory intravenous dosage regimen of ciprofloxacin, computed on the basis of disposition kinetic data obtained in healthy buffalo calves, would be 3 mg/kg repeated at 12 h intervals.

Keywords: buffalo calves, ciprofloxacin, disposition kinetics, dosage excretion, fluoroquinolone, urine

Abbreviations: C_{max}, peak plasma concentration; HPLC, high-performance liquid chromatography; MEC, minimum effective concentration; and see legend to Table I

INTRODUCTION

Ciprofloxacin, a fluoroquinolone antibacterial, is employed effectively in humans and animals in the treatment of several bacterial infections, such as those with *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Haemophilus ducreyi*, and various strains of *Salmonella*, *Shigella*, *Enterobacter*, *Campylobacter* and *Neisseria* (Sanders, 1988). Even certain intracellular bacteria-like organisms, such as *Chlamydia*, *Mycoplasma*, *Brucella* and *Mycobacterium*, are inhibited by ciprofloxacin (Leysen *et al.*, 1989). Infections with these organisms are manifested in buffalo by diarrhoea, pneumonia, pneumoenteritis (Khan and Khan, 1997), mastitis (Galiero *et al.*, 1996), repeat breeding (Dhabale *et al.*, 1997), sexually transmitted diseases (Modolo *et al.*, 1999), chlamydiosis (Baldi *et al.*, 1999) and tuberculosis (Kanameda

et al., 1997), indicating that ciprofloxacin might be used clinically in buffaloes. Disposition kinetic studies of antimicrobial agents, which provide a basis for the determination of satisfactory dosage regimens, are required in all animal species in which the drugs are to be used clinically (Gibaldi and Perrier, 1982). The disposition kinetics of ciprofloxacin have been investigated in humans (Hoffken *et al.*, 1985), cows (Kaartinen *et al.*, 1994), sheep (Munoz *et al.*, 1996), dogs (Abadia *et al.*, 1994) and ponies (Dowling *et al.*, 1995), but no work has been reported for buffalo. In view of the marked species variations in the kinetic data for antimicrobial drugs, the present study was undertaken to determine the disposition kinetics and urinary excretion of ciprofloxacin in buffalo calves after a single intravenous administration. An appropriate dosage regimen of ciprofloxacin in buffalo species was also then to be derived based on the kinetic parameters.

MATERIALS AND METHODS

Experimental animals and drug administration

Five healthy male buffalo calves weighing 70–120 kg were used. The animals were adapted to the standard laboratory conditions and feeding schedule for 1 month prior to the commencement of the experiments. Water was supplied *ad libitum*. The animals were used for studying both the disposition kinetics and urinary excretion simultaneously. After cannulae had been inserted into both the jugular veins of each animal, they were moved to metabolic stalls of standard size. Ciprofloxacin (Ciprolet, Dr Reddy's Laboratories Ltd., Bollaram, Medak, India), as 2% ciprofloxacin lactate, was injected into the left jugular vein of each animal at a dose of 5 mg/kg body weight.

Blood and urine sampling

Blood samples (5 ml) were collected from the contralateral jugular vein into heparinized glass centrifuge tubes before and at 1, 2.5, 5, 7.5, 10, 15, 30 and 45 min and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 20 h after administration of the drug. The plasma was separated after centrifugation at 2000*g* for 15 min at room temperature and stored at –20°C until analysis, usually on the next day. The metabolic stalls were designed so that, along with the collection of blood samples, all the urine voided by the animal can be collected at the base of the stall over the chosen time intervals, without contamination or spillage. The urine voided by the animals was collected at 2, 4, 8, 12, 18 and 24 h following administration of the drug, its volume was measured and samples (10 ml) were stored at –20°C until analysis.

Analytical procedure

The concentration of ciprofloxacin-equivalent inhibitory units in the plasma and urine was determined by a microbiological assay technique (Arret *et al.*, 1971) using *Escherchia coli* (ATCC 25922) as the test organism. The minimum detection level was 0.02 μg/ml. The various disposition kinetic parameters in each animal were calculated using a nonlinear iterative curve-fitting computer program, STATIS Version 3 (M/s Clydesoft, Glasgow, UK) and also by the computed least-squares technique (Gibaldi and Perrier, 1982).

Computation of dosage regimen

Using a convenient dosage interval, the computation of the maintenance dose was based on the minimum effective concentration (MEC) and calculated using

$$D = C_p\,(\text{min})^{\infty} \times V_{d(\text{area})} \times (e^{\beta\tau} - 1)$$

where β is the elimination rate constant and τ is the dosing interval. The priming dose was calculated by omitting the –1 from the above equation.

RESULTS

After the single IV administration, the mean concentrations of ciprofloxacin in the plasma (as ciprofloxacin-equivalent inhibitory units), plotted on a semilogarithmic scale as a function of time, are as presented in Figure 1. At 1 min, the peak plasma concentration was 8.50 ± 0.39 μg/ml, which rapidly decreased to 1.45 ± 0.04 μg/ml at 1 h. Thereafter, the concentration of ciprofloxacin-equivalent inhibitory units fell gradually and only traces (0.02 ± 0.0 μg/ml) were detected at 20 h after dosing. The pharmacokinetic parameters that describe the distribution and elimination of ciprofloxacin in buffalo calves are given in Table I. Data on urinary excretion of ciprofloxacin in buffalo calves are presented in Table II. Taking 12 h as a suitable dosage interval, the dosage regimen of ciprofloxacin was computed for various MEC_{90} and is presented in Table III.

DISCUSSION

Küng and colleagues (1993) have reported that the microbiological assay method is comparable to HPLC for estimating concentrations of fluoroquinolones up to 1000 ng/ml. Hoffken and colleagues (1985) reported that, in humans, the estimation of ciprofloxacin concentrations by microbial assay was 3–27% more sensitive than could be measured by HPLC. Hence, a microbial assay method was preferred over HPLC in the present study for computing the dosage regimen for ciprofloxacin, which is

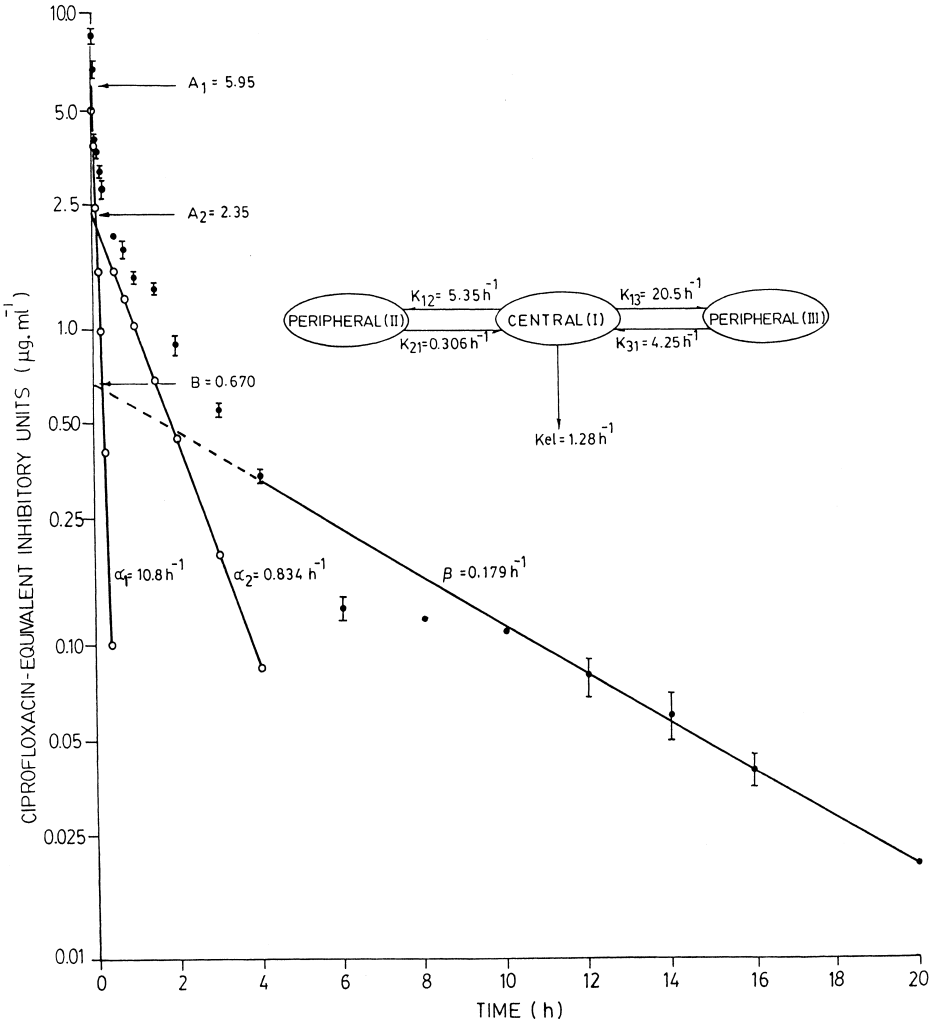

Figure 1. A semilogarithmic plot of the plasma concentration–time profile of ciprofloxacin in buffalo calves (n = 5) following a single intravenous dose of 5 mg/kg body weight. The values are presented as mean ± SEM. The data were analysed by a three-compartment open model. Distribution (α_1 and α_2) and elimination (β) phases are represented by least-squares regression lines

metabolized in the body to the active metabolites desethylene ciprofloxacin, sulfociprofloxacin and oxociprofloxacin (Frost *et al.*, 1989).

The peak plasma concentration of ciprofloxacin-equivalent inhibitory units found in this study is in accordance with the findings of Bergeron (1989), who reported the C_{max} of all fluoroquinolones to be in the range 1.5–10.7 μg/ml. An average concentration of

TABLE I
Disposition kinetics of ciprofloxacin in buffalo calves (n = 5) after single intravenous administration of 5 mg/kg body weight

Parameter[a]	Unit	Mean ± SEM
C_p^0	μg/ml	8.97 ± 0.38
A_1	μg/ml	5.95 ± 0.45
A_2	μg/ml	2.35 ± 0.41
B	μg/ml	0.670 ± 0.071
α_1	h^{-1}	10.8 ± 1.64
α_2	h^{-1}	0.834 ± 0.083
β	h^{-1}	0.179 ± 0.004
$t_{\frac{1}{2}\alpha_1}$	h	0.071 ± 0.010
$t_{\frac{1}{2}\alpha_2}$	h	0.885 ± 0.061
$t_{\frac{1}{2}\beta}$	h	3.88 ± 0.08
K_{12}	h^{-1}	5.35 ± 0.30
K_{13}	h^{-1}	20.5 ± 2.39
K_{21}	h^{-1}	0.306 ± 0.006
K_{31}	h^{-1}	4.25 ± 0.88
K_{el}	h^{-1}	1.28 ± 0.10
$t_{\frac{1}{2}}K_{el}$	h	0.554 ± 0.044
Cl_B	L/kg per h	0.709 ± 0.025
$AUC_{(0-\infty)}$	(μg/ml) h	7.12 ± 0.23
AUMC	(μg/ml) h^2	24.5 ± 2.44
$V_{d(area)}$	L/kg	3.97 ± 0.22
$V_{d(B)}$	L/kg	7.68 ± 0.68
$V_{d(ss)}$	L/kg	2.40 ± 0.07
V_c	L/kg	0.56 ± 0.022
MRT	h	3.41 ± 0.14
F_c	ratio	0.143 ± 0.011
T/P	ratio	6.13 ± 0.54

[a]The kinetic parameters are as described by Gibaldi and Perrier (1982)

C_p^0 = plasma drug concentration at time zero after intravenous dose; A_1, A_2 = zero-time intercept of distribution phases I and II, respectively; B = zero-time intercept of elimination phase; α_1, α_2 = distribution rate constants of phases I and II, respectively; β = overall elimination rate constant; $t_{\frac{1}{2}\alpha_1}$, $t_{\frac{1}{2}\alpha_2}$ = half-lives of distribution phases I and II, respectively; $t_{\frac{1}{2}\beta}$ = elimination half-life; K_{12}, K_{13} = rate of transfer of drug from central to peripheral compartments I and II, respectively; K_{21}, K_{31} = rate of transfer of drug from peripheral compartments I and II, respectively into central compartment; K_{el} = elimination rate constant from central compartment; $t_{\frac{1}{2}}K_{el}$ = elimination half-life from central compartment; Cl_B = total body clearance of the drug; $AUC_{(0-\infty)}$, AUMC = area under plasma concentration–time curve and its first moment, respectively; $V_{d(area)}$, $V_{d(B)}$, $V_{d(ss)}$ = volume of distribution from $AUC_{(0-\infty)}$, elimination phase and steady-state plasma level, respectively; V_c = volume of central compartment; MRT = mean residence time; F_c = fraction of dose in central compartment; T/P = tissue/plasma ratio of drug concentration

TABLE II
Urinary concentration and cumulative excretion of ciprofloxacin from buffalo calves (n = 5) after a single intravenous administration of 5 mg/kg body weight

Excretion interval (h)	Concentration (μg/ml)	Cumulative percentage of total dose excreted
0–2	79.0 ± 4.21	9.46 ± 0.59
2–4	55.4 ± 1.66	16.6 ± 1.70
4–8	50.3 ± 7.85	21.6 ± 1.99
8–12	24.4 ± 3.43	25.4 ± 1.86
12–18	21.2 ± 12.6	27.1 ± 2.10
18–24	4.25 ± 0.97	28.3 ± 2.28

Values are expressed as mean ± SEM

TABLE III
Computed intravenous dosage regimen of ciprofloxacin in buffalo calves (n = 5) required to maintain specified concentrations in plasma (MEC_{90})

	Dosage interval (h)		
MEC_{90} (μg/ml)	8	12	16
0.02	0.33 (0.25)	0.68 (0.60)	1.39 (1.31)
0.04	0.66 (0.51)	1.36 (1.20)	2.78 (2.63)
0.06	1.0 (0.76)	2.04 (1.80)	4.18 (3.94)
0.08	1.33 (1.01)	2.72 (2.40)	5.57 (5.25)
0.10	1.66 (1.27)	3.40 (3.00)	6.96 (6.56)
0.12	1.99 (1.52)	4.08 (3.61)	8.35 (7.88)
0.14	2.33 (1.77)	4.76 (4.21)	9.74 (9.19)
0.16	2.66 (2.02)	5.44 (4.81)	11.1 (10.5)
0.18	2.99 (2.28)	6.12 (5.41)	12.5 (11.8)
0.20	3.32 (2.53)	6.80 (6.01)	13.9 (13.1)

Values given are priming dose (maintenance dose) and are expressed as mg/kg body weight

$<$0.2 μg/ml has been reported to be the minimum therapeutic plasma concentration of fluoroquinolones in general (Norris and Mandell, 1988) and ciprofloxacin in particular (Vance-Bryan *et al.*, 1990). Keeping in mind the synergistic effect of the immune system and other *in vivo* factors, and to cover most of the susceptible organisms, 0.10 μg/ml was chosen as the minimum effective concentration. This was maintained for up to 10 h after administration. The C_{max} to MIC ratio achieved was 85.0, which is much higher than the value (8–10) suggested by some workers (Lode *et al.*, 1998; Meinen *et al.*, 1995).

The evaluation of the plasma concentration–time curve revealed a triphasic decline, so the data were best fitted to a three-compartment open model and adequately described by

$$C_p = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t} + B e^{-\beta t}$$

where C_p is the concentration at time t, A_1, A_2 and B are the intercept terms of distribution phases I and II and the elimination phase, respectively, and α_1, α_2 and β are the rate constants for the distribution phases I and II and the elimination phase, respectively. Ciprofloxacin was also reported to follow a three-compartment open model in human beings (Hoffken *et al.*, 1985).

The high values for α_1 and for the transfer rate K_{13} suggest that ciprofloxacin was rapidly distributed in the various body fluids and tissues of the buffalo calves. The value of the distribution rate constant of ciprofloxacin was reported to be 13.9 h^{-1} in dogs (Abadia *et al.*, 1994). The high values for $V_{d(area)}$ and the T/P ratio (tissue to plasma ratio of drug concentration) showed that ciprofloxacin was extensively distributed in the extravascular space. This is in accordance with the findings of Alestig (1990), who reported that, in contrast to β-lactams, fluoroquinolones achieve tissue concentrations many times higher than that in the plasma. Smaller values for the $V_{d(area)}$ of ciprofloxacin have been reported in dogs (3 L/kg; Abadia *et al.*, 1994) and ponies (3.45 L/kg; Dowling *et al.*, 1995). The elimination half-life of ciprofloxacin found in the present study was similar to that reported in humans (3–4 h; Hoffken *et al.*, 1985), but higher than that reported in dogs (3.0 h; Abadia *et al.*, 1994), ponies (2.63 h; Dowling *et al.*, 1995) and sheep (1.2 h; Munoz *et al.*, 1996).

The value of Cl_B found in the present investigation was higher than that reported in dogs (0.468 ± 0.094 L/kg per h; Cester and Toutain, 1997) but lower than that reported in ponies (1.09 ± 0.24 L/kg per h; Dowling *et al.*, 1995). The value for the $AUC_{(0-\infty)}$ (area under the plasma concentration versus time curve) of ciprofloxacin found in the present investigation was similar to that found in humans (7.06 ± 1.21 (μg/ml) h; Hong *et al.*, 1995). The $AUC_{(0-\infty)}$ to MIC ratio observed in this investigation was 71.2. Nightingale and colleagues (2000) have suggested that, for effective eradication and a good clinical outcome, the AUC:MIC ratio of fluoroquinolones should be >30 for Gram-positive and >100 for Gram-negative bacteria. Accordingly, the observed ratio covers all the Gram-positive and most Gram-negative bacteria. The value for the mean residential time of ciprofloxacin found in the present investigation was lower than that found in dogs (4.20 ± 0.82 h; Cester and Toutain, 1997). This was expected since the Cl_B of ciprofloxacin in the present study was about 34% more than that in dogs.

The concentration of ciprofloxacin-equivalent inhibitory units in the urine was very high, being more than 40 times the MEC (minimum effective concentration) even 24 h after administration. Within 24 h, approximately 28.3% of total administered dose had been recovered in the urine. These findings suggest that ciprofloxacin may be an appropriate drug for treating urinary tract infections in buffalo. Hong and colleagues (1995) have reported that 60.9% of ciprofloxacin is excreted in urine in humans. The recovery of large amounts of the drug in the urine of buffalo calves within 12 h of its intravenous administration (25.4%) corresponds well with the decline in the concentra-

tion of the drug in the plasma during this phase. It was reported earlier that ciprofloxacin is eliminated by both the renal (glomerular filtration and tubular secretion) and non-renal (hepatic and transintestinal) routes (Blum, 1992).

The ultimate objective of the disposition kinetic study was to determine appropriate IV dosage regimens for ciprofloxacin in buffalo. Taking different dosage intervals for maintaining various minimum therapeutic plasma levels (Norris and Mandell, 1988; Vance-Bryan *et al.*, 1990) and using the values of β and $V_{d(area)}$ from Table I, an appropriate dosage regimen of ciprofloxacin in buffalo calves (Table III) infected with organisms sensitive to the drug would be 3.40 mg/kg followed by 3.00 mg/kg at 12 h intervals.

Although, in the present study, the usual MEC for ciprofloxacin of 0.10 μg/ml was only maintained in the plasma for 10 h after an IV injection of 5 mg/kg body weight, the lower dose (i.e. 3 mg/kg at 12 h intervals) is appropriate since the dosage of fluoroquinolones in tissue infections should not only be related to the concentrations in the plasma, because they achieve concentrations in the tissues many times higher than those in the plasma (Alestig, 1990).

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