

Pharmacokinetics of cefpirome in buffalo calves (*Bubalus bubalis*) following single intramuscular administration

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Summary

The pharmacokinetics of cefpirome was investigated in buffalo calves following its single intramuscular (IM) administration (10 mg). The peak plasma concentration of cefpirome at 30 min was $9.0 \pm 0.5 \mu\text{g ml}^{-1}$, which declined to $0.2 \pm 0.1 \mu\text{g ml}^{-1}$ at 24 hrs. The absorption half-life ($t_{1/2\text{Ka}}$) and elimination half-life ($t_{1/2\beta}$) were 0.19 ± 0.03 hr and 2.39 ± 0.05 hr, respectively. The area under the plasma concentration-time curve (AUC), area under the first moment curve (AUMC), apparent volume of distribution ($V_{d\text{area}}$), total body clearance (Cl_B), mean residence time (MRT) and total duration of therapeutic effect (td) were $28.7 \pm 1.9 \mu\text{g.ml}^{-1}.\text{hr}$, $107.7 \pm 6.7 \mu\text{g.ml}^{-1}.\text{hr}^2$, $0.42 \pm 0.01 \text{ L.kg}^{-1}$, $0.12 \pm 0.003 \text{ L.kg}^{-1}.\text{hr}^{-1}$, 3.76 ± 0.04 hr and 12.7 ± 0.3 hr, respectively. The systemic bioavailability (F) after IM administration of cefpirome in calves was $35.3 \pm 3.1\%$. To maintain a minimum therapeutic concentration of $0.25 \mu\text{g.ml}^{-1}$, a satisfactory dosage regimen of cefpirome in buffalo calves was 3.46 mg.kg^{-1} followed by 3.36 mg.kg^{-1} at 12-hour intervals. Cefpirome was bound to the plasma proteins of buffalo calves *in vitro* to the extent of $30.7 \pm 1.9\%$.

Key words: Buffalo, Cefpirome, Cephalosporins, Dosage, Pharmacokinetics

Introduction

Cefpirome (HR 810) is a new cephalosporin with a 2,3-cyclopentenopyridine group in the 3-position side chain. It has potent bactericidal activity against a broad range of gram-negative and gram-positive organisms including *Pseudomonas aeruginosa* and methicillin-susceptible staphylococcus species and it is also highly active against *Haemophilus influenzae* type b and many members of the family Enterobacteriaceae (Clarke *et al.*, 1985). It has low affinity for β -lactamase (Kobayashi *et al.*, 1986) hence it is more resistant to inactivation by β -lactamase-producing bacteria. The disposition kinetics of cefpirome has been investigated in human beings, rats, mice, rabbits, dogs, guinea pigs and monkeys (Isert *et al.*, 1992; Kita *et al.*, 1992; Mrestani *et al.*, 2003; Saemann *et al.*, 2005). Such

data are absolutely lacking in buffalo calves. In animal studies, it shows high blood levels with a prolonged half-life (Klesel and Seeger, 1983). Keeping in view, the significant species variation in pharmacokinetic behaviour of antibiotics (Sharma *et al.*, 2004), the present study was planned to investigate the disposition kinetics and *in vitro* plasma protein binding of cefpirome in buffalo calves. From the pharmacokinetic parameters, a derived guidance was made for an optimal dosage regimen of cefpirome in buffalo calves.

Materials and Methods

Experimental animals and drug administration

The experiment was performed in 5 healthy male buffalo calves, 6–12-month-old and weighing 90–122 kg. The animals were adapted to laboratory conditions for 2 weeks

prior to the commencement of the study and were provided with green fodder and water *ad libitum*. Cefpirome (Cefpirom, Orchid Chemicals and Pharmaceuticals Ltd, Ahmedabad, India) was administered intramuscularly (IM) at a dose of 10 mg.kg^{-1} body weight in the lateral neck region.

Collection of samples

Blood samples were withdrawn from the jugular vein into heparinized glass centrifuge tubes at 1, 2.5, 5, 7.5, 10, 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14 and 24 hrs after administration of drug. The plasma was separated by centrifugation at 1300 g at room temperature and stored at -20°C until analysis, which usually took place on the day after collection.

Bioassay of the drug

The concentration of cefpirome in the plasma was determined by a standard microbiological bioassay technique (Arret *et al.*, 1971) using *Escherichia coli* (MTCC 739) as the test organism. The test organism was streaked on a sterilized slant of antibiotic medium No. 1 and incubated at 37°C for 24 hrs. The washing of the slant in 3 ml normal saline was transferred into a Roux bottle containing 250 ml of sterilized solidified medium No. 1 which was incubated at 37°C for 24 hrs. A suspension was prepared of the resultant bacterial growth in 50 ml sterile normal saline and stored under refrigeration. Assay plates were prepared by putting 25 ml of seed layer of medium No. 11 poured on 100 ml capacity assay Petri dishes. Seed layer was made by adding a desired amount of bacterial suspension to obtain optimum bacterial growth and the required dimensions of zone of inhibition with a reference concentration ($0.25 \text{ } \mu\text{g.ml}^{-1}$) of cefpirome. Preliminary experiments were conducted to determine the actual amount of bacterial suspension to be used in the preparation of seed layer. Six wells were punctured at equal distance by a punching machine after solidification of the media. The alternate three wells were filled with plasma sample and the remaining three wells with a standard solution of cefpirome ($0.25 \text{ } \mu\text{g.ml}^{-1}$). These assay plates were incubated at 32°C for 6 hrs. At the end of

incubation, the diameter of zone of inhibition of each well was measured with a Fisher Lilly Antibiotic Zone Reader (Fisher Scientific, New Jersey, USA). For each sample, 9 replicates were analysed. This assay could detect a minimum of $0.05 \text{ } \mu\text{g.ml}^{-1}$ of cefpirome. The standard curve of cefpirome was prepared by adding different concentrations of the drug from 0.05 to $0.5 \text{ } \mu\text{g.ml}^{-1}$ in plasma of buffalo calves (Fig. 1).

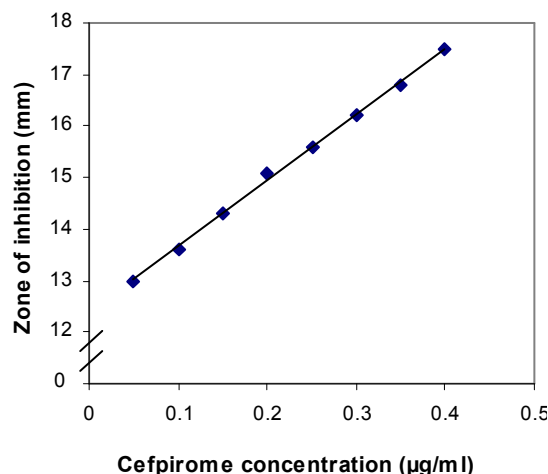


Fig. 1: Standard curve of cefpirome in plasma of buffalo calves. Each point represents the mean of results from 12 assays

Pharmacokinetic variables and dosage regimen

Pharmacokinetic parameters were calculated by the computed least-square linear regression technique (Gibaldi and Perrier, 1982). Different estimates of the volume of distribution were obtained from the following equations:

$$V_{d_{\text{area}}} = \frac{\text{Dose (mg.kg}^{-1}\text{)}}{\beta \cdot \text{AUC}}$$

$$V_{d_B} = \frac{\text{Dose (mg.kg}^{-1}\text{)}}{B}$$

$$V_{d_{ss}} = \frac{\text{Dose (mg.kg}^{-1}\text{)} \cdot \text{AUMC}}{\text{AUC}^2}$$

The priming (D) and maintenance doses (D') of cefpirome, at various dosage intervals, for maintaining different MICs was calculated from the following equations:

$$D = \text{Cp}(\text{min})^{\infty} \cdot V_{d_{\text{area}}} \cdot (e^{\beta\tau})$$

$$D' = \text{Cp}(\text{min})^{\infty} \cdot V_{d_{\text{area}}} \cdot (e^{\beta\tau} - 1)$$

Where β is the elimination rate constant and τ is the dosage interval.

Plasma protein binding

In vitro binding of cefpirome to plasma proteins was determined by employing the equilibrium dialysis technique (Kunin *et al.*, 1959). The various concentrations of cefpirome .g. 1, 10, 20, 50 and 100 $\mu\text{g}.\text{ml}^{-1}$ were prepared in plasma taken from untreated animals. Each bag filled with 5 ml of plasma containing known amount of drug was then immersed in a separate tube containing 5 ml of phosphate buffer and the tubes were incubated at 37°C for 24 hrs with occasional shaking. At the end of incubation period buffer as well as contents of the dialysing bags were separately analysed for the concentration of cefpirome. For each concentration three separate sets of experiments were conducted. The extent of *in vitro* plasma protein binding of cefpirome was calculated by the following equation:

$$\text{Per cent of cefpirome bound to plasma protein} = \frac{C_p - C_B}{C_p} \times 100$$

Where C_p is the concentration of cefpirome in plasma after incubation, C_B , concentration of cefpirome in phosphate buffer after incubation and C_p , concentration of cefpirome in plasma before incubation.

Results

The standard curve of plasma cefpirome concentration versus zone of inhibition was straight between 0.05 and 0.4 $\mu\text{g}.\text{ml}^{-1}$ drug concentration and the coefficient of variation was 4.31. The plasma levels of cefpirome at different time intervals are presented in Fig. 2. The plasma concentration of cefpirome at 1 min after a single IM injection was $0.24 \pm 0.01 \mu\text{g}.\text{ml}^{-1}$, which gradually increased and the peak plasma concentration ($9.04 \pm 0.5 \mu\text{g}.\text{ml}^{-1}$) was observed at 30 min. The drug levels above the minimum inhibitory concentration (MIC) were detected in plasma up to 12 hrs. The pharmacokinetic parameters that describe the absorption and elimination pattern of cefpirome were calculated and are presented in Table 1. Taking various dosage intervals, the different desired plasma concentrations

ranging from 0.1 to 0.5 $\mu\text{g}.\text{ml}^{-1}$ and using the values for β and $V_{d\text{area}}$ from Table 1, the necessary doses of cefpirome were calculated and are presented in Table 2. Table 3 summarizes the parameters of *in vitro* plasma protein binding of cefpirome. At plasma concentrations of 1 to 100 $\mu\text{g}.\text{ml}^{-1}$ the extent of plasma protein binding of cefpirome ranged from 39.3 to 24.2% with an overall mean of $30.7 \pm 1.9\%$.

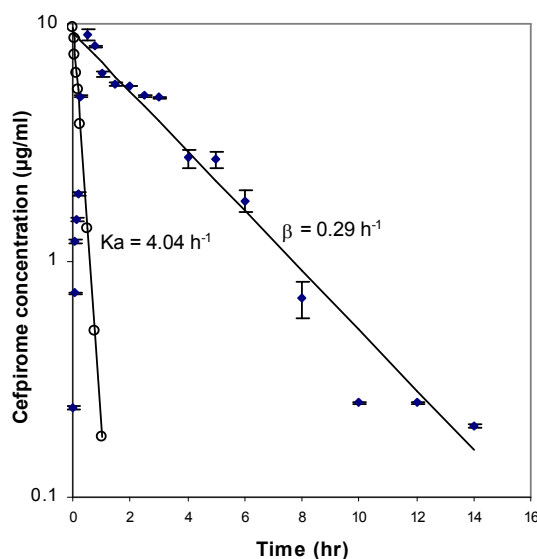


Fig. 2: Semilogarithmic plot of plasma concentration-time profile of cefpirome in healthy buffalo calves following a single intramuscular dose of 10 $\text{mg}.\text{kg}^{-1}$ body weight. Values given are mean \pm SE ($n = 5$). Data was analysed according to one-compartment open model. Absorption (K_a) and elimination (β) phases are represented by least square regression lines. The calculated points (o) of the absorption phase were obtained by the residual method

Discussion

The evaluation of the results on observed plasma levels of cefpirome indicated that the data can be best fitted to one-compartment open model and pharmacokinetics was described by the equation: $C_p = Be^{-\beta t} - A'e^{-K_{at}}$. Mono-compartment model has been used to describe the disposition pattern of cefuroxime, cefoperazone and cefotaxime in buffalo calves and for cefotaxime in endometritic buffaloes after IM administration (Agnihotri *et al.*, 2002; Sharma *et al.*, 2004; Goyal *et al.*, 2005; Singh *et al.*, 2005).

Table 1: Pharmacokinetic parameters of ceftiofime in buffalo calves after a single intramuscular dose of 10 mg.kg⁻¹ body weight

Pharmacokinetic parameter	Calf number					Mean ± SE
	1	2	3	4	5	
A' (μg.ml ⁻¹)	10.1	13.1	10.7	8.42	9.30	10.3 ± 0.8
B (μg.ml ⁻¹)	9.10	12.3	9.84	6.77	8.09	9.23 ± 0.9
Ka (hr ⁻¹)	3.72	2.47	3.25	6.32	4.44	4.04 ± 0.7
β (hr ⁻¹)	0.28	0.31	0.30	0.28	0.28	0.29 ± 0.01
t _{1/2Ka} (hr)	0.19	0.28	0.21	0.11	0.16	0.19 ± 0.03
t _{1/2β} (hr)	2.48	2.24	2.31	2.48	2.48	2.39 ± 0.05
AUC (μg.ml ⁻¹ .hr)	29.8	34.5	29.5	22.8	26.8	28.7 ± 1.9
AUMC (μg.ml ⁻¹ .hr ²)	115.3	126.2	108.3	86.1	102.7	107.7 ± 6.7
Vd _{area} (L.kg ⁻¹)	0.43	0.42	0.42	0.41	0.43	0.42 ± 0.01
Cl _B (L.kg ⁻¹ .hr ⁻¹)	0.12	0.13	0.13	0.11	0.12	0.12 ± 0.003
MRT (hr)	3.87	3.66	3.67	3.77	3.83	3.76 ± 0.04
td (hr)	13.2	11.9	12.3	13.2	13.2	12.7 ± 0.3
C _{max} (μg.ml ⁻¹)	10.0	8.13	10.2	8.08	8.75	9.04 ± 0.5
t _{max} (hr)	0.50	0.50	0.50	0.50	0.50	0.5 ± 0.00
C _{max} /MIC (ratio)	40.0	32.5	40.8	32.3	35.0	36.1 ± 1.8
AUC/MIC (ratio)	119.1	137.8	118.0	91.4	107.2	114.7 ± 7.6
F (%)	35.9	44.8	37.2	26.0	32.5	35.3 ± 3.1

A' and B, zero-time plasma concentration intercepts of regression lines of absorption and elimination phases, respectively; Ka and β, absorption and elimination coefficients, respectively, in the mono-exponential equation that describes the plasma concentration-versus-time data; t_{1/2Ka} and t_{1/2β}, half-lives of absorption and elimination phases, respectively; AUC, area under the plasma concentration-time-curve; AUMC, area under the first moment of the plasma concentration-time-curve; Vd_{area}, volume of distribution from AUC; Cl_B, total body clearance of the drug; MRT, mean residence time of drug in body; td, total duration of pharmacological effect, C_{max}, maximum plasma concentration; t_{max}, time required to attain peak plasma level; F, per cent of drug available in the central compartment after extravascular administration

Table 2: Doses of ceftiofime (mg.kg⁻¹) at various intervals for different MIC in buffalo calves

MIC (μg.ml ⁻¹)	Dose	Dosage interval (hr)			
		8	10	12	16
0.1	D	0.43	0.77	1.39	4.46
	D'	0.39	0.73	1.35	4.42
0.2	D	0.86	1.55	2.77	8.92
	D'	0.78	1.46	2.69	8.83
0.25	D	1.08	1.93	3.46	11.2
	D'	0.97	1.83	3.36	11.1
0.3	D	1.30	2.32	4.15	13.4
	D'	1.17	2.19	4.03	13.3
0.5	D	2.16	3.87	6.93	22.3
	D'	1.95	3.65	6.72	22.1

D = priming dose and D' = maintenance dose

Table 3: *In vitro* binding and kinetic constants of binding of ceftiofime to plasma proteins of buffalo calves

Exp. No	Extent of binding					Association rate constant, β_i (mole.g ⁻¹)	Dissociation rate constant, K_β (mole)
	Concentration of cefpirome ($\mu\text{g.ml}^{-1}$)						
	1	10	20	50	100		
1	38.0	34.5	28.9	26.1	29.0	2.41×10^{-8}	3.77×10^{-7}
2	43.0	40.0	26.7	31.2	21.1	1.75×10^{-8}	1.13×10^{-6}
3	37.0	33.2	30.8	17.9	22.5	2.48×10^{-8}	3.18×10^{-7}
Mean \pm SE	39.3	35.9	28.8	25.1	24.2	2.21×10^{-8}	6.08×10^{-7}
	± 1.9	± 2.1	± 1.2	± 3.9	± 2.5	$\pm 0.23 \times 10^{-8}$	$\pm 2.61 \times 10^{-7}$

Overall mean ± SE of extent (%) of binding = 30.7 ± 1.9

IM injection resulted into appreciable plasma concentration of ceftiofame at 1 min, which attained the peak at 30 min. Similar to our findings, Goyal *et al.* (2005) reported peak plasma concentration ($8.1 \mu\text{g.ml}^{-1}$) of cefoperazone after 30 min of IM injection in buffalo calves. Following IM administration, the mean peak plasma concentration of $11.3 \mu\text{g. ml}^{-1}$ at 20 min in buffalo calves and $8.0 \mu\text{g.ml}^{-1}$ at 30 min in endometritic buffaloes reported for cefotaxime support the results of the present study (Sharma *et al.*, 2004; Singh *et al.*, 2005).

The rapid appearance of drug in plasma indicated fast systemic absorption following IM injection. This was further confirmed by the high value of absorption rate constant ($4.04 \pm 0.7 \text{ hr}^{-1}$) and short absorption half-life ($0.19 \pm 0.03 \text{ hr}$). Rapid absorption following IM injection has also been reported for cefoperazone (Goyal *et al.*, 2005), cefotaxime (Sharma *et al.*, 2004) and cefuroxime (Agnihotri *et al.*, 2002).

The high value of AUC ($28.7 \pm 1.91 \mu\text{g.ml}^{-1}.\text{hr}$) and AUMC ($107.7 \pm 6.7 \mu\text{g.ml}^{-1}.\text{hr}^2$) obtained after IM administration of ceftiofame in buffalo calves indicated vast area of body covered under drug concentration. Almost similar value of AUC ($21.7 \mu\text{g.ml}^{-1}.\text{hr}$) was reported in buffalo calves after IM administration of cefotaxime, another third generation cephalosporin commonly used in veterinary practice (Singh *et al.*, 2005). The value of Vd_{area} ($0.42 \pm 0.01 \text{ L.kg}^{-1}$) after IM injection of ceftiofame in the present study is in accordance with the reports of other cephalosporins. However, higher value of Vd_{area} (1.30 L.kg^{-1}) has been reported for cefotaxime following IM administration in buffalo calves (Sharma and Srivastava, 2003).

The elimination of ceftiofame was rapid with a $t_{1/2\beta}$ of $2.39 \pm 0.05 \text{ hr}$ following IM administration in buffalo calves. Similar to our findings low values of $t_{1/2\beta}$ for ceftiofame ranging from 0.4 hr in rats to 1.1 hr in dogs were reported (Isert *et al.*, 1992). Further, low values of $t_{1/2\beta}$ (1.24 hr) of cefotaxime (Sharma *et al.*, 2004) and 1.56 hr of cefuroxime (Agnihotri *et al.*, 2002) were obtained in buffalo calves following IM injection. The total body clearance (Cl_B) of

ceftiofame in the present study was $0.12 \pm 0.003 \text{ L.kg}^{-1}.\text{hr}^{-1}$. Similar value of Cl_B was reported for cefoperazone $0.16 \text{ L.kg}^{-1}.\text{hr}^{-1}$ after IM administration in buffalo calves (Goyal *et al.*, 2005).

The low value of systemic bioavailability ($35.3 \pm 3.1\%$) in the present study indicated limited absorption of drug from the IM injection site. For other cephalosporins, however, high value of bioavailability after IM injection were reported as 86.7% for cefotaxime (Dardi *et al.*, 2004) in buffalo calves.

The priming and maintenance IM doses of ceftiofame in buffalo calves with a dosage interval of 12 hrs were 3.46 and 3.36 mg.kg^{-1} , respectively, or in field condition it would be 3.5 mg.kg^{-1} to be repeated at 12-hour intervals in buffalo calves.

Ceftiofame was bound to the plasma proteins of buffalo calves to the extent of $30.7 \pm 1.9\%$. The plasma protein binding of ceftiofame in buffalo calves was concentration dependent being greater at lower concentrations of the drug. However, ceftiofame has been reported to be 8.8% bound to protein in rat granuloma pouch exudate (Arai *et al.*, 1988). Similarly, Mayer *et al.* (2000), Herkner *et al.* (2002) and Muller *et al.* (1997) have reported that ceftiofame was bound to the plasma proteins to the extent of 10% in human beings. The value of β_i and K_β ranged between 1.75×10^{-8} to $2.48 \times 10^{-8} \text{ mol.g}^{-1}$ and 1.13×10^{-6} to $3.77 \times 10^{-7} \text{ mol}$, respectively, in the present study, reflecting that the binding of ceftiofame to the plasma proteins of buffalo calves is weak and reversible.

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