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Pharmacokinetics after single intramuscular administration and *in vitro* plasma protein binding of cefoperazone in cross bred calves

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ABSTRACT

The present study was conducted to investigate the pharmacokinetics of cefoperazone after single intramuscular (i/m) administration of 20 mg.kg^{-1} into the lateral neck region and its *in vitro* plasma protein binding in male cross bred calves. The concentration of cefoperazone in plasma samples was estimated by a standard microbiological assay technique using *Escherichia coli* (ATCC 10536) as the test organism. Appreciable plasma concentration of cefoperazone (1.14 ± 0.07 μg.mL^{-1}) was detected at 1 min after injection and the peak plasma level of 9.76 ± 0.25 μg.mL^{-1} was observed at 45 min. The drug level above MIC_{90} in plasma, was detected up to 5 h of administration. Rapid absorption of the drug was also evident by the short absorption half-life (0.55 ± 0.08 h). The overall systemic bioavailability of cefoperazone after intramuscular administration was 48.1 ± 5.33%. The high value of AUC (15.7 ± 0.64 μg.mL^{-1}.h) reflected a vast area of body covered by drug concentration. Extensive distribution of the drug into various body fluids and tissues was reflected by the high value of the steady state volume of distribution (2.95 ± 0.28 L.kg^{-1}). The elimination half-life and MRT were 2.31 ± 0.05 h and 3.62 ± 0.06 h, respectively. The total body clearance (Cl_B) was 1.28 ± 0.05 L.kg^{-1}.h^{-1}. Cefoperazone was bound to the plasma proteins of calves to the extent of 24.9 ± 1.11%. There was no statistically significant difference in the pharmacokinetic parameters calculated by compartmental and non-compartmental analysis except the values of AUC, AUMC and Cl_B. On the basis of the pharmacokinetic parameters, an appropriate i/m dosage regimen for cefoperazone in calves would be 26 mg. kg^{-1} followed by 22 mg.kg^{-1} at 6 h intervals.

Key words: calves, cefoperazone, pharmacokinetics, protein binding

Introduction

Cefoperazone, a broad spectrum third generation cephalosporin, possesses activity against gram-positive, gram-negative and anaerobic bacteria including *H. influenzae,*

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Neisseria meningitidis and *Streptococcus pneumoniae.* Cefoperazone is suitable for the treatment of bone and joint infections of horses (SORACI et al., 1998), intensive care infections of human beings (FANTON et al., 1987) and has good penetration in the pancreas indicating its usefulness in prophylaxis and therapy of secondary pancreatic infections (JIANG et al., 1997). Clinical studies have demonstrated cefoperazone to be a valuable drug in the treatment of calf diseases such as diarrhoea and pneumonia associated with gram-negative bacteria resistant to many commonly used antibiotics (SOBACK et al., 1986). The pharmacokinetics of cefoperazone has been determined in unweaned (SOBACK and ZIV, 1989) and weaned (GUPTA et al., 2007) calves, buffalo calves (GOYAL et al., 2003 and 2005), horses (SORACI et al., 1996), sheep (GUERRINI et al., 1985) and human beings (DANZIGER et al., 1994).

However, such data is lacking after extravascular administration of cefoperazone in calves. In view of the marked species variation in the pharmacokinetic data of antimicrobial drugs, the present study was undertaken to determine the pharmacokinetics and an appropriate dosage regimen, following single intramuscular administration and *in vitro* plasma protein binding of cefoperazone in cross bred calves.

Materials and methods

Four healthy male cross bred calves (Holstein Friesian×Sahiwal), less than one year of age and weighing between 88-120 kg were kept under identical conditions of management in the departmental animal shed and were maintained on green fodder and wheat straw. Water was provided *ad libitum.* All the animals were healthy at the time of experimentation. The experimental protocol followed the ethical guidelines on the proper care and use of animals. Cefoperazone (Panacea Biotech Ltd., India) was administered i/m into the lateral neck region at the dose rate of 20 $mg.kg^{-1}$ body mass as a freshly prepared 20% solution. The dose of the drug employed in the present study was comparable to the doses used in calves and other species of animals by previous workers (CARLI et al., 1986; GOYAL et al., 2003 and 2005; MARINO et al., 1987; SOBACK and ZIV, 1989). Blood samples (5 mL) were withdrawn from the jugular vein into heparinized glass centrifuge tubes before and 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 and 12 h after administration of drug. Plasma was separated by centrifugation at 1300 g for 15 min at room temperature and kept at -20 ^{0}C until analysis, which was done usually on the day after collection. The concentration of cefoperazone in plasma samples was estimated by the microbiological assay technique (ARRET et al., 1971) using *Escherichia coli* (ATCC 10536) as the test organism. The assay plates were incubated at 37 ^{0}C for 12 h. At the end of incubation, the diameter of the zone of inhibition of each well was measured with a Fisher Lilly Antibiotic Zone Reader (Fisher Scientific Company USA). For each sample, 9 replicates were analysed and compared with the zone of inhibition of reference solution of cefoperazone (0.3 $\mu g.mL^{-1}$). The concentration of cefoperazone in the samples was

calculated as μg.mL^{-1} of plasma or urine. The assay detected a minimum of 0.25 μg.mL^{-1} of cefoperazone without differentiating between the parent drug and its metabolites.

In vitro binding of cefoperazone to plasma proteins was determined by employing the equilibrium dialysis technique (KUNIN et al., 1959) and the constants for protein binding were obtained. Various concentrations of cefoperazone (3.125 to 50 μg.mL^{-1}) were prepared in pooled plasma taken from untreated animals. Each dialyzing bag (4 Aº pore size) filled with 5 mL of plasma containing a known amount of drug was then immersed in a separate tube containing 5 mL of phosphate buffer (0.2 M; pH 7.4) and the tubes were incubated at 37 ºC for 24 h with occasional shaking. At the end of the incubation period buffer as well the contents of the dialyzing bags were separately analysed for the concentration of cefoperazone. For each concentration three separate sets of experiments were conducted. The extent of *in vitro* plasma protein binding of cefoperazone was calculated by the following equation:

$$\text{Per cent of cefoperazone bound to plasma protein} = \frac{CP^1 - CB}{CP} \times 100$$

where, CP^1 is the concentration of cefoperazone in the plasma after incubation, CB is the concentration of cefoperazone in the phosphate buffer after incubation and CP is the concentration of cefoperazone in the plasma before incubation. Pharmacokinetic parameters were calculated manually by the computed least-squares linear regression technique by applying compartmental and non-compartmental analysis (GIBALDI and PERRIER, 1982). The mean pharmacokinetic variables were obtained by averaging the variables calculated for drug disposition after i/m drug administration to each animal. Overall systemic bioavailability was calculated by using the values of AUC and β obtained after single intravenous administration of cefoperazone (GUPTA et al., 2007) in the same animals, which were used for i/m study of cefoperazone with a washing period of 30 days. The difference between the two means of the individual observations was determined by the Student's *t*-test. The significance was assessed at $P<0.05$ and $P<0.01$ levels.

Results

The mean plasma levels of cefoperazone in crossbred calves, at various time intervals, after a single i/m administration (20 mg.kg^{-1}) are presented on a semilogarithmic scale in Figure 1. Intramuscular injection resulted in the appreciable plasma concentration of drug (1.14 ± 0.07 μg.mL^{-1}) at 1 min and peak plasma level of 9.76 ± 0.25 μg.mL^{-1} was attained at 45 min post administration. The drug was detected in plasma for up to 8 h after dosing (0.54 ± 0.02 μg.mL^{-1}). Evaluation of the results revealed that the disposition pattern of cefoperazone was adequately described by the bi-exponential equation: $C_p = Be^{-\beta t} - A'e^{-\alpha t}$ where, C_p is the plasma level of cefoperazone at time t and e represents

Table 1. Pharmacokinetic parameters of cefoperazone in cross bred calves (n = 4) following single intramuscular dose of 20 mg.kg^{-1} body mass

Parameter	Unit	Mean ± SE	
		Compartmental analysis	Non-compartmental analysis
A'	μg.mL^{-1}	2.36 ± 0.43	-
α	h^{-1}	1.37 ± 0.26	-
B	μg.mL^{-1}	5.28 ± 0.25	-
β	h^{-1}	0.301 ± 0.006	-
C_{max}	μg.mL^{-1}	9.76 ± 0.25	-
t_{max}	min	45.0 ± 0.0	-
C_{max}/MIC	ratio	9.76 ± 0.25	-
$t_{1/2\alpha}$	h	0.55 ± 0.08	0.90 ± 0.27
$t_{1/2\beta}$	h	2.31 ± 0.05	2.32 ± 0.11
AUC	μg.mL^{-1}.h	15.7 ± 0.64	20.5 ± 0.37**
AUMC	μg.mL^{-1}.h^2	56.6 ± 1.62	68.5 ± 3.47*
Vd_{area}	L.kg^{-1}	4.26 ± 0.19	4.22 ± 0.35
Vd_{ss}	L.kg^{-1}	2.95 ± 0.28	3.28 ± 0.17
Cl_B	mL.kg^{-1}.h^{-1}	1.28 ± 0.05	0.98 ± 0.02 **
MRT	h	3.62 ± 0.06	3.35 ± 0.27
F	%	48.1 ± 5.33	56.7 ± 6.03

A' and B = zero-time plasma drug concentration intercepts of the regression lines of absorption and elimination phases, respectively; α and β = absorption and elimination rate constants, respectively; C_{max} and t_{max} = peak plasma drug concentration and time required to attain the peak concentration, respectively; MIC = minimum inhibitory concentration of drug in plasma; $t_{1/2\alpha}$ = absorption half-life; $t_{1/2\beta}$ = elimination half-life; AUC = area under the plasma concentration-time curve; AUMC = area under the first moment curve; Vd_{area}, Vd_{ss} = apparent volume of distribution based on AUC and steady state plasma levels, respectively; Cl_B = total body clearance; MRT = mean residence time; F = overall systemic bioavailability. Signicant difference *(P<0.05), **(P<0.01)

Table 2. *In vitro* binding and kinetic constants of binding of cefoperazone to plasma proteins of calves

Exp. N^o	Extent of binding					Association rate constant, β_i (mole.g^{-1})	Dissociation rate constant, K_β (mole)
	Concentration of cefoperazone (μg.mL^{-1})						
	3.125	6.25	12.5	25	50		
1	22.7	26.4	25.4	21.3	27.8	1.81×10^{-8}	5.88×10^{-7}
2	21.2	27.7	15.5	22.2	28.0	9.63×10^{-9}	1.68×10^{-6}
3	30.4	31.8	24.0	21.3	28.4	2.95×10^{-8}	6.57×10^{-8}
Mean ± SE	24.8 ± 2.85	28.6 ± 1.63	21.6 ± 3.09	21.6 ± 0.30	28.1 ± 0.18	1.91×10^{-8} ± 5.75×10^{-9}	7.77×10^{-7} ± 4.79×10^{-7}

Values given are expressed as percentage of drug bound with plasma proteins. Overall Mean ± SE of extent (%) of binding = 24.9 ± 1.11

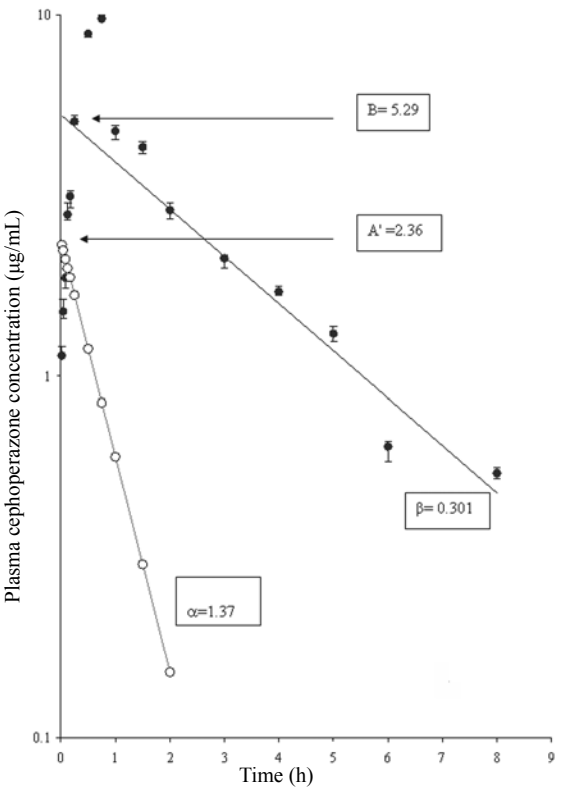

Fig. 1. Semilogarithmic plot of plasma concentration-time profile of cefoperazone following its single intramuscular injection of 20 mg.kg^{-1} body mass in cross bred calves. Values are presented as mean ± SE of 4 animals. The data was analysed according to one-compartment open model. Absorption and elimination phases are represented by least square regression lines. The calculated points (o) of absorption phases were obtained by residual method. Constants A' and B are zero-time intercepts of absorption and elimination phases, respectively.

the base of the natural logarithm. A' and B are the extrapolated zero-time intercepts of the absorption and elimination phases, respectively. α and β are the rate constants of the absorption and elimination phases, respectively. The pharmacokinetic parameters that describe the disposition pattern of cefoperazone in calves, were calculated manually and are presented in Table 1.

The plasma concentration-time data of cefoperazone was also subjected to non-compartmental analysis based on statistical moment theory (Table 1). Table 2 summarizes the parameters of *in vitro* plasma protein binding of cefoperazone. At plasma

concentrations of 3.125 to 50 μg.mL^{-1} the extent of plasma protein binding of cefoperazone ranged from 21.6 to 28.6% with an overall mean of 24.9 ± 1.11%. Using a convenient dosage interval and the values of β and Vd_{area} from Table 1, the priming (D) and maintenance (D') doses of cefoperazone were calculated from following equations: $D = C_p min^{\propto}.Vd\ (e^{\beta\tau})$, $D' = C_p min^{\propto}.\ Vd\ (e^{\beta\tau}-1)$ where, $C_p min^{\propto}$ is the MIC of cefoperazone, β is the elimination rate constant and τ is the dosing interval (BAGGOT, 1977).

Discussion

The pharmacokinetics of cefoperazone following i/m administration in calves, best fitted the one-compartment open model. The rapid appearance of cefoperazone in plasma following its i/m administration and the high value of $t_{1/2\alpha}$ suggested that the drug rapidly entered into systemic circulation. The plasma level of ≥ 0.2 μg.mL^{-1} for third generation cephalosporins is considered adequate against most species of sensitive bacteria, including enterobacteriaceae spp. (BARRIERE and FLAHERTY, 1984). However, a plasma concentration of 0.25-2.0 μg.mL^{-1} has been reported as the minimum inhibitory concentration (MIC_{90}) of cephalosporins against common animal pathogens (CRAIG, 1998). In this discussion, an average value of MIC (1.0 μg.mL^{-1}) has been taken into consideration. The drug was detected above MIC in plasma up to 5 h of administration.

The large steady-state volume of distribution in the present study (2.95 ± 0.28 L.kg^{-1}) which was more than its corresponding value of 0.713 L.kg^{-1} in unweaned calves (SOBACK and ZIV, 1989) indicated extensive distribution of cefoperazone into various body fluids and tissues. The high value of AUC (15.7 ± 0.64 μg.mL^{-1}.h) in the present study reflected that a vast area was covered by cefoperazone concentration. On the basis of AUC and β after single i.v. (29.0 ± 1.03 μg.mL^{-1}.h and 0.349 ± 0.035 h^{-1}, respectively) and i/m administration in calves (Table 1), the systemic bioavailability of cefoperazone was calculated to be 48.1 ± 5.33% in the present study which was comparable to 42% in horses (SORACI et al., 1998), but lower than 76.3% in unweaned calves (SOBACK and ZIV, 1989) observed after i/m administration of cefoperazone. The Cl_B of cefoperazone in the present study was 1.28 ± 0.05 L.kg^{-1}.h^{-1} indicating rapid clearance of the drug in cross bred calves. In contrast, low value of Cl_B (489.6 mL.kg^{-1}.h^{-1}) has been reported following i/m administration of cefoperazone in unweaned calves (SOBACK and ZIV, 1989). The $t_{1/2\beta}$ of cefoperazone in calves in this study (2.31 ± 0.05 h) was comparable to the $t_{1/2\beta}$ of 2.28 h in unweaned calves (SOBACK and ZIV, 1989), 1.6-2.4 h in human beings (CRAIG and GERBER, 1981) and 1.52 h in horses (SORACI et al., 1998).

Comparison of compartmental and non-compartmental analysis did not reveal much statistically significant difference in the pharmacokinetic parameters calculated by the two methods except the values of AUC and AUMC which were slightly higher and Cl_B which

was slightly lower when calculated by non-compartmental analysis. Cefoperazone was bound to the plasma proteins of calves the extent of 24.9 ± 1.11%. However higher values have been reported for the plasma protein binding of cefoperazone in calves (44%) and human beings (90-95%) in earlier studies (BARRIERE and FLAHERTY, 1984; CARLI et al., 1986; CRAIG and GERBER, 1981). The negligible value of the binding capacity of plasma proteins (β_i) and high value of dissociation rate constant (K_β) in the present study, reflected that the binding of cefoperazone to the plasma proteins of calves was weak and reversible. On the basis of the present study, for most bacteria sensitive to cefoperazone, the most appropriate dosage regimen would be 26 mg.kg^{-1} to be repeated by 22 mg.kg^{-1} at 6 h intervals in calves under field conditions.

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SAŽETAK

Istražena je farmakokinetika cefoperazona nakon jednokratne intramuskularne primjene u dozi od 20 $mg.kg^{-1}$ u vratno mišićje i njegovo *in vitro* vezanje na proteine plazme u križane muške teladi. Koncentracija cefoperazona u uzorcima plazme bila je određena standardnim mikrobiološkim postupkom rabeći bakteriju *Escherichia coli* (ATCC 10536). Mjerljiva koncentracija cefoperazona u plazmi ($1,14 \pm 0,07$ $\mu g.mL^{-1}$) dokazana je jednu minutu nakon ubrizgavanja, a vršna razina od $9,76 \pm 0,25$ $\mu g.mL^{-1}$ ustanovljena je nakon 45 minuta. Koncentracija iznad MIC90 u plazmi ustanovljena je do 5. sata nakon ubrizgavanja. Brza apsorpcija lijeka bila je također vidljiva po brzom poluvremenu apsorpcije ($0,55 \pm 0,08$ sati). Ukupna sistemna bioraspoloživost cefoperazona nakon intramuskularne primjene iznosila je $48,1 \pm 5,33\%$. Visoka vrijednost područja ispod krivulje (AUC) ($15,7 \pm 0,64$ $\mu g.mL^{-1}.h$) bila je odraz velikoga područja tijela pod antibiotikom. Opsežna raspodjela lijeka u različitim tjelesnim tekućinama i tkivima bila je odraz visoke vrijednosti postojanoga volumena raspodjele ($2,95 \pm 0,28$ $L.kg^{-1}$). Poluvrijeme izlučivanja iznosilo je $2,31 \pm 0,05$, a srednje vrijeme održavanja $3,62 \pm 0,06$ sati. Ukupan tjelesni klirens (ClB) bio je $1,28 \pm 0,05$ $L.kg^{-1}.h^{-1}$. Cefoperazon se vezao na proteine plazme teladi u rasponu od $24,9 \pm 1,11\%$. Nije ustanovljena statistički značajna razlika u farmakokinetičkim pokazateljima analizom po pojedinim odjeljcima, osim za vrijednosti područja ispod krivulje (AUC), područja ispod krivulje netom nakon primjene (AUMC) i ukupnog tjelesnog klirensa. Na temelju farmakokinetičkih pokazatelja, prikladni režim doziranja cefoperazona primijenjenog i/m u teladi bio bi 26 $mg.kg^{-1}$, a potom po 22 $mg.kg^{-1}$ u razmacima od 6 sati.

Ključne riječi: telad, cefoperazon, farmakokinetika, vezanje na proteine