VETERINARSKI ARHIV 82 (1), 1-9, 2012

Disposition kinetics and *in vitro* plasma protein binding of cefpirome in cattle

Neetu Rajput, Vinod K. Dumka*, and Harpal S. Sandhu

Department of Pharmacology and Toxicology College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India

RAJPUT, N., V. K. DUMKA, H. S. SANDHU: Disposition kinetics and *in vitro* plasma protein binding of cefpirome in cattle. Vet. arhiv 82, 1-9, 2012.

ABSTRACT

The disposition of cefpirome after single intramuscular (i.m.) administration (10 $mg.kg^{-1}$) was investigated in five male cross-bred calves and *in vitro* plasma protein binding was determined. The concentration of cefpirome in the plasma was estimated by the microbiological assay technique. Binding of cefpirome to plasma proteins was determined at different concentration levels by the equilibrium dialysis technique. The peak plasma level of cefpirome after i.m. administration to cattle was attained at 45 min post-dose and the drug was detected in plasma above MIC of 0.5 $\mu g.mL^{-1}$ for up to 10 h. The drug disposition followed a one-compartment open model. The values of $t_{1/2Ka,}$ $t_{1/2\beta}$ and AUC were 0.21 ± 0.01 h, 2.06 ± 0.02 h and 31.7 ± 0.95 $\mu g.mL^{-1}.h$, respectively. Cefpirome was bound to the plasma proteins to the extent of 26.0 ± 2.84 percent at the concentration range of 1-100 $\mu g.mL^{-1}$. The binding capacity of cefpirome to plasma proteins and the dissociation rate constant of the protein-drug complex were $3.71 \times 10^{-8} \pm 0.31 \times 10^{-8}$ $mole.g^{-1}$ and $3.43 \times 10^{-7} \pm 0.46 \times 10^{-7}$ mole, respectively.

Key words: calves, cefpirome, disposition, protein binding

Introduction

Cephalosporins are among the most widely used group of antibacterials in veterinary and human medical practice. Cefpirome, a new fourth generation cephalosporin, possesses an extended spectrum of activity against resistant organisms such as *Pseudomonas* spp. (SULTANA and ARAYNE, 2007; OZBEK and OTUK, 2010). It is commonly used in humans to treat bacterial meningitis and infections of the lower respiratory tract, skin and urinary tract (PRESCOTT, 2006). Cefpirome has the clinical advantage of being highly stable to hydrolysis by several β-lactamases including Amp C β-lactamases that confer resistance to a wide variety of β-lactam antibiotics and, therefore, it is clinically useful

*Corresponding author:

Dr. V. K. Dumka, M.V.Sc., Ph.D, Associate Professor, Department of Pharmacology and Toxicology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India, Phone: +91 161 2414 032; (M) +91 946 3201 126; Fax: +91 161 2400 822; E-mail: vkdumka@yahoo.com

ISSN 0372-5480
Printed in Croatia

against organisms producing Amp C-type β-lactamases (PRESCOTT, 2006; AHMED and SHIMAMOTO, 2008). In view of the species variation in the disposition kinetic data of antimicrobials, it is considered important to investigate the disposition kinetics of drugs in different animal species and under different environmental conditions. The disposition kinetics of cefpirome have been described in rabbits (MRESTANI et al., 2003), rats (ISERT et al., 1992), dogs (ISERT et al., 1992; KITA et al., 1992), monkeys (KLESEL and SEEGER, 1983), buffalo calves (RAJPUT et al., 2007a,b; RAJPUT et al., 2008) and humans (SAUERMANN et al., 2005). It has been emphasized that the pharmacokinetic data of a drug generated in one species cannot be extrapolated to other species. Since there is no information available on the pharmacokinetic behaviour of cefpirome in cross-bred calves, the present study was undertaken to investigate the disposition kinetics after single intramuscular administration and *in vitro* plasma protein binding of cefpirome in cross-bred calves.

Materials and methods

Experimental animals and drug administration. The study was conducted on five healthy male cross-bred calves between 6-12 months of age and weighing 80-120 kg. The animals were dewormed and kept under observation for 2 weeks of acclimatization before commencement of the experiment. During the experimental period, the animals were maintained on concentrate feed and were allowed to graze freely. Water was provided *ad libitum*. The average daily temperature in the shed was about 25 °C during the experimental period. The experimental protocol followed the ethical guidelines on the proper care and use of animals and was approved by the Institutional Animal Ethics Committee. Cefpirome (Cepirom, Orchid Chemicals and Pharmaceuticals Ltd, India) was administered by a single intramuscular (i.m.) injection into the lateral neck region at the dose rate of 10 $mg.kg^{-1}$.

Sample collection. Blood samples (6 mL) were collected into heparinized glass centrifuge tubes by jugular venipuncture at time intervals of 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 h of administration of cefpirome. Plasma was separated and stored at -20 °C until analyzed for cefpirome, which was usually done on the next day after collection.

Analytical method. The concentration of cefpirome in plasma was estimated by the microbiological assay technique (ARRET et al., 1971) using *Escherichia coli* (microbial type culture collection; MTCC 739) as the test organism, as detailed in previously published reports (RAJPUT et al., 2007b). This method detected both the parent compound and its active metabolites. The assay could detect a minimum of 0.05 $\mu g.mL^{-1}$ of the antimicrobial active compound.

Pharmacokinetic analysis. The concentrations of cefpirome in the plasma were plotted on a semi-logarithmic scale as a function of time and the pharmacokinetic parameters were

calculated manually for each animal by the least square regression technique (GIBALDI and PERRIER, 1982).

Plasma protein binding. In vitro binding of cefpirome to plasma proteins was determined by employing the equilibrium dialysis technique (KUNIN et al., 1959). Various concentrations of cefpirome (1, 10, 20, 50 and 100 μg.mL^{-1}) were prepared in plasma taken from untreated cattle. Each dialyzing bag filled with 5 mL of plasma, containing a known amount of the drug, was then immersed in separate tubes containing 5 mL of phosphate buffer and the tubes were incubated at 37 °C for 24 h with occasional shaking. At the end of the incubation period, the buffer as well as the contents of the dialysing bags, were analyzed separately 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 equation:

$$\text{Percent of cefpirome bound to plasma protein} = \frac{CP' - CB}{CP} \times 100$$

Where, CP$'$ is the concentration of cefpirome in the plasma after incubation, CB is the concentration of cefpirome in the phosphate buffer after incubation and CP is the concentration of cefpirome in the plasma before incubation. The constants for binding of cefpirome to plasma proteins were obtained as described by PILLOUD (1973) to describe the capacity and nature of the binding characteristics of the drug.

Results

The plasma levels of cefpirome at different time intervals are presented in Fig. 1. The plasma concentration of cefpirome at 1 min after a single i.m. injection was 0.31 ± 0.01 μg.mL^{-1}, and it gradually increased until the peak plasma concentration (10.1 ± 0.05 μg.mL^{-1}) was observed at 45 min. The pharmacokinetic parameters that described the absorption and elimination pattern of cefpirome were calculated and are presented in Table 1. Table 2 summarizes the parameters of *in vitro* plasma protein binding of cefpirome.

Table 1. Disposition kinetic parameters of cefpirome in cross-bred claves (n = 5) after a single intramuscular dose of 10 mg.kg^{-1} body weight

Parameter	Animal number					Mean ± SE
	1	2	3	4	5	
A$^{'}$ (μg.mL^{-1})	14.4	12.8	13.7	12.7	12.2	13.1 ± 0.40
B (μg.mL^{-1})	13.2	11.6	12.6	11.2	11.2	12.0 ± 0.39
Ka (h^{-1})	3.09	3.40	3.13	3.77	3.19	3.32 ± 0.13
β (h^{-1})	0.33	0.34	0.35	0.33	0.33	0.34 ± 0.01
$t_{½Ka}$ (h)	0.22	0.20	0.22	0.18	0.22	0.21 ± 0.01
$t_{1/2\,\beta}$ (h)	2.10	2.04	1.98	2.10	2.10	2.06 ± 0.02
AUC (μg.mL^{-1}.h)	35.3	30.5	31.7	30.7	30.3	31.7 ± 0.95
AUMC (μg.mL^{-1}.h^2)	119.7	99.6	101.6	102.3	102.0	105.1 ± 3.69
MRT (h)	3.39	3.27	3.21	3.33	3.37	3.31 ± 0.03
C_{max} (μg.mL^{-1})	10.1	10.1	9.98	10.2	10.3	10.1 ± 0.05
t_{max} (h)	0.75	0.75	0.75	0.75	0.75	0.75 ± 0.00

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 $t_{1/2\beta}$, 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; MRT, mean residence time of drug in body; $C_{max,}$ maximum plasma concentration; t_{max}, time required to attain peak plasma level.

Table 2. *In vitro* binding and kinetic constants of binding of cefpirome to plasma proteins of cross-bred calves (n = 5)

Exp. No.	Extent of binding (%) Concentration of cefpirome (μg.mL^{-1})					Association rate constant, β_i (mole.g^{-1})	Dissociation rate constant, K_β (mole)
	1	10	20	50	100		
1	47.0	19.1	25.2	17.6	23.8	3.96 ×10^{-8}	4.34 ×10^{-7}
2	48.0	17.7	22.8	18.7	25.8	4.07 ×10^{-8}	2.98 ×10^{-7}
3	29.0	41.9	12.2	21.7	19.7	3.09 ×10^{-8}	2.96 ×10^{-7}
Mean ± SE	41.3 ± 6.17	26.2 ± 7.84	20.1 ± 3.99	19.3 ±1.25	23.1 ± 1.80	3.71 ×10^{-8} ± 0.31 ×10^{-8}	3.43 ×10^{-7} ± 0.46 ×10^{-7}

Overall Mean ± SE of extent of binding = 26.0 ± 2.84%.

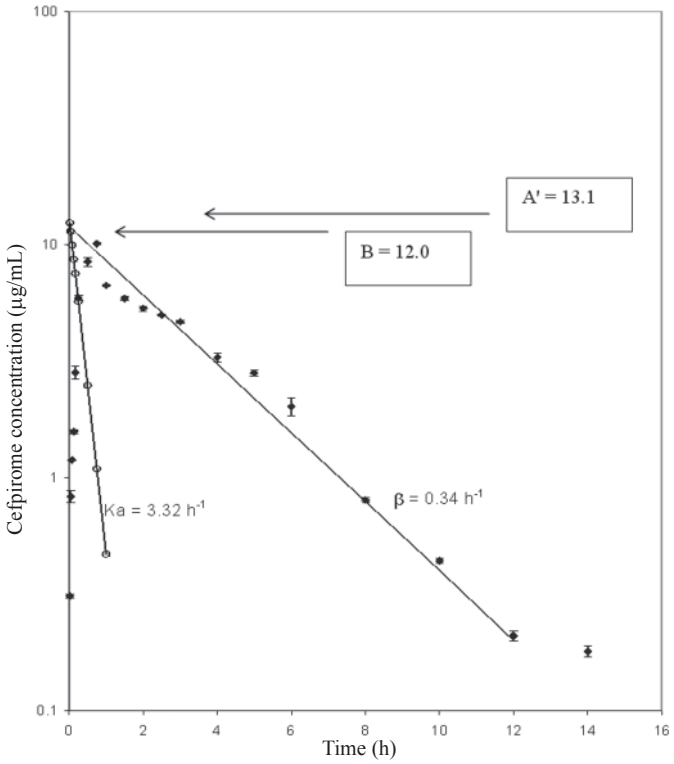

Fig. 1. Semilogarithmic plot of plasma concentration-time profile of cefpirome in cross-bred calves following a single intramuscular dose of 10 mg.kg^{-1} body weight. Values given are mean ± SE of 5 animals. Data was analyzed according to the one-compartment open model. Absorption (Ka) and elimination (β) phases are represented by the 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 the observed plasma levels of cefpirome indicated that the data may be best fitted to the one-compartment open model and the pharmacokinetics were described by the equation: $C_p = Be^{-\beta t} - A'e^{-kat}$. The mono-compartment model has also been used to describe the disposition pattern of cefpirome (RAJPUT et al., 2007b) and ceftriaxone (GOHIL et al., 2009) in buffalo calves and ceftizoxime in calves (SINGH et al., 2008) following single i.m. administration. The rapid attainment of peak cefpirome concentration in plasma suggested that this drug rapidly entered into systemic circulation

following i.m. administration. A similar peak plasma level of cefpirome (9.04 $\mu g.mL^{-1}$) was produced at 0.5 h after i.m. administration in buffalo calves (RAJPUT et al., 2007b). The minimum therapeutic plasma concentration of cefpirome against human pathogens has been reported to be 0.05-0.39 $\mu g.mL^{-1}$ (ARAI et al., 1988) but such values for cefpirome have not been determined against animal isolates. Another fourth-generation cephalosporin, cefquinome, is active against pathogens generally found in animal infections and possesses a potency and spectrum comparable to cefpirome (MURPHY et al., 1994). The minimum inhibitory concentration (MIC_{90}) of cefquinome against animal pathogens, including *E. coli*, *Klebsiella pneumoniae, Haemophilus influenzae, Pasteurella, Salmonella* and *Streptococcus* species, have been reported in the range of 0.03-1.0 $\mu g.mL^{-1}$ (AL-TAHER, 2010). In this study, a concentration of 0.5 $\mu g.mL^{-1}$ was considered as the reference plasma level for cefpirome.

The study of kinetic determinants of cefpirome following i.m. administration revealed a low value of absorption half-life, $t_{½ka}$ (0.21 ± 0.01 h) in the present study, which was similar to the low $t_{½ka}$ of 0.19 h reported for cefpirome in buffalo calves (RAJPUT et al., 2007b), further confirming that after i.m. administration, absorption of cefpirome is very rapid. Rapid absorption after i.m. injection has also been reported for cefepime and ceftizoxime in crossbred calves (ISMAIL, 2005; SINGH et al., 2008) and ceftriaxone in buffalo calves (GOHIL et al., 2009). The high values of AUC (31.7 ± 0.95 $\mu g.mL^{-1}.h$) AUMC (105.1 ± 3.69 $\mu g.mL^{-1}.h^2$) in the present study were comparable to the values of AUC (28.7 ± 1.9 $\mu g.mL^{-1}.h$) and AUMC (107.7 ± 6.7 $\mu g.mL^{-1}.h^2$) of cefpirome in buffalo calves (RAJPUT et al., 2007b). Similarly, high values of AUC have also been reported in dogs (103 $\mu g.mL^{-1}.h$), rabbits (18.9 $\mu g.\ min.mL^{-1}$) and man (16.5 $g.min.L^{-1}$) for cefpirome (KITA et al., 1992; MRESTANI et al., 2003; SAUERMANN et al., 2005).

The elimination of cefpirome was rapid, with a $t_{½\beta}$ of 2.06 ± 0.02 h following i.m. administration in crossbred calves. Short elimination half-lives have also been reported for cefpirome after i.v. (2.14 h) and i.m. (2.39 h) administration and cefepime (3.0 h) following i.v. administration in buffalo calves (RAJPUT et al., 2007a,b; JOSHI and SHARMA, 2009) and for ceftizoxime (1.44 h) and cefepime (3.02 h) after i.m. administration in calves (ISMAIL, 2005; SINGH et al., 2008). The value of MRT in crossbred calves following i.m. administration of cefpirome (3.31 ± 0.03 h) in the present study was in accordance with the values of MRT (3.76 h) observed after i.m. administration of cefpirome in buffalo calves (RAJPUT et al., 2007b), however it was longer than the MRT of ceftriaxone (1.55 h) reported after intravenous (i.v.) administration in cattle (KUMAR et al., 2010).

The extent of plasma protein binding of cefpirome varied from 12.2 to 48.0 percent with an overall mean of 26.0 ± 2.84 percent. The calculated values of the capacity of cefpirome to bind with plasma proteins of crossbred calves (β_i) and the dissociation rate constant of protein drug complex (K_β) were $3.71 \times 10^{-8} \pm 0.31 \times 10^{-8}$ $mol.g^{-1}$ and $3.43 \times 10^{-7} \pm$

0.46 $\times 10^{-7}$ mol, respectively. The binding of the drug to plasma proteins was weak and reversible, which was evident from the lower value of β_i than the value of K_β. These results are in accordance with the 30.7% binding of cefpirome to the plasma proteins of buffalo calves (RAJPUT et al., 2007b), however a low value of plasma protein binding of cefpirome has been reported at 10 percent in humans (STEINER et al., 2004) and 8.8 percent in rats (ARAI et al., 1988). A marked inter-species difference has been reported in the binding of cephalosporins to plasma proteins in animals. A plasma protein binding study of six cephalosporins in animals revealed high protein binding in rat, monkey, and human plasma, whereas in dog plasma, protein binding was markedly lower (RICHTER et al., 1998). Similarly the plasma protein binding of cefoperazone in calves has been reported to be nearly one fourth of that in humans (GUPTA et al., 2008).

Acknowledgements

The financial assistance received by the senior author from Guru Angad Dev Veterinary and Animal Sciences University, in the form of a University Merit Scholarship to pursue the present study, is duly acknowledged.

References

AHMED, A. M., T. SHIMAMOTO (2008): Emergence of a cefepime- and cefpirome-resistant *Citrobacter freundii* clinical isolate harbouring a novel chromosomally encoded AmpC β-lactamase, CMY-37. Int. J. Antimicrob. Agents. 32, 256-261.

AL-TAHER, A. Y. (2010): Pharmacokinetics of cefquinome in camels. J. Anim. Vet. Adv. 9, 848-852.

ARRET, B., D. P. JOHNSON, A. KIRSHBAUM (1971): Outline of details for microbiological assay of antibiotics: second revision. J. Pharm. Sci. 60, 1689-1694.

ARAI, S., S. KOBAYASHI, S. HAYASHI, T. SAKAGUCHI (1988): Distribution of cefpirome (HR 810) to exudate in the croton-oil-induced rat granuloma pouch and its therapeutic effects on experimental infections in the pouch. Antimicrob. Agents Chemother. 32, 1396-1399.

GIBALDI, M., D. PERRIER (1982): Methods of residuals. In: Pharmacokinetics. 2nd ed. Marcel Dekker, New York. pp. 433-444.

GOHIL, P. V., U. D. PATEL, S. K. BHAVSAR, A. M. THAKER (2009): Pharmacokinetics of ceftriaxone in buffalo calves (*Bubalus bubalis*) following intravenous and intramuscular administration. Iranian J. Vet. Res. 10, 33-37.

GUPTA, J. K., R. K. CHAUDHARY, V. K. DUMKA (2008): Pharmacokinetics after single intramuscular administration and *in vitro* plasma protein binding of cefoperazone in cross bred calves. Vet. Arhiv 78, 441-448.

ISERT, D., N. KLESEL, M. LIMBERT, A. MARKUS, G. SEIBERT, E. SCHRINNER (1992): Pharmacokinetics of cefpirome administered intravenously or intramuscularly to rats and dogs. J. Antimicrob. Chemother. 29, 31-37.

ISMAIL, M. M. (2005): Disposition kinetics, bioavailability and renal clearance of cefepime in calves. Vet. Res. Commun. 29, 69-79.

JOSHI, B., S. K. SHARMA (2009): The pharmacokinetics of cefepime in *E. coli* lipopolysaccharide induced febrile buffalo calves. Vet. Arhiv 79, 523-530.

KITA, Y., T. YAMAAKI, A. IMADA (1992): Comparative pharmacokinetics of SCE-2787 and related antibiotics in experimental animals. Antimicrob. Agents Chemother. 36**,** 481-486.

KLESEL, N., K. SEEGER (1983): Pharmacokinetic properties of the new cephalosporin antibiotic HR 810 in animals. Infection 11, 318-321.

KUMAR, S., A. K. SRIVASTAVA, V. K. DUMKA, N. KUMAR, R. K. RAINA (2010): Plasma pharmacokinetics and milk levels of ceftriaxone following single intravenous administration in healthy and endometritic cows Vet. Res. Commun. 34, 503-510.

KUNIN, C. M., R. J. DORNBUS, M. FINLAND (1959): Distribution and excretion of tetracycline analogies in normal young man. J. Clin. Invest. 38, 1950-1963.

MRESTANI, Y., B. BRETSCHNEIDER, A. HARTI, R. H. H. NEUBERT (2003): *In-vitro* and *in-vivo* studies of cefpirome using bile salts as absorption enhancers. J. Pharm. Pharmacol. 55, 1601-1606.

MURPHY, S. P., M. E. ERWIN, R. N. JONES (1994): Cefquinome (HR 111V) *in vitro* evaluation of a broad-spectrum cephalosporin indicated for infections in animals. Diag. Microbiol. Infect. Dis. 20, 49-55.

OZBEK, B., G. OTUK (2010): Activities of fourth generation cephalosporins alone and in combination with gentamicin, amikacin, ciprofloxacin and levofloxacin against bloodstream *Pseudomonas aeruginosa* isolates. Ind. J. Med. Microbiol. 28, 84-85.

PILLOUD, M. (1973): Pharmacokinetics, plasma protein binding and dosage of oxytetracycline in cattle and horses. Res. Vet. Sci. 15, 224-230.

PRESCOTT, J. F. (2006): Beta lactam antibiotics: cephalosporins. In: Antimicrobial Therapy in Veterinary Medicine, 4th ed. (Giguere, S., J. F. Prescott, J. D. Baggot, Eds.), Wiley-Blackwell, San Francisco. pp.139-158.

RAJPUT, N., V. K. DUMKA, H. S. SANDHU (2007a): Disposition kinetics and urinary excretion of cefpirome after intravenous injection in buffalo calves. J. Vet. Sci. 8, 21-25.

RAJPUT, N., V. K. DUMKA, H. S. SANDHU (2007b): Pharmacokinetic disposition of cefpirome in buffalo calves (*Bubalus bubalis*) following single intramuscular administration. Iranian J. Vet. Res. 8, 212-217.

RAJPUT, N., V. K. DUMKA, H. S. SANDHU (2008): Influence of experimentally induced fever on the disposition of cefpirome in buffalo calves. Environ. Toxicol. Pharmacol. 26, 305-308.

RICHTER, W. F., P. HEIZMANN, J. MEYER, V. STARKE, T. LAVE (1998): Animal pharmacokinetics and interspecies scaling of Ro 25-6833 and related (lactamylvinyl) cephalosporins. J. Pharm. Sci. 87, 496-500.

SAUERMANN, R., G. DELLE-KARTH, C. MARSIK, I. STEINER, M. ZEITLINGER, B.X. MAYER-HELM, A. GEORGOPOULOS, M. MULLER, C. JOUKHADAR (2005):

Pharmacokinetics and pharmacodynamics of cefpirome in subcutaneous adipose tissue of septic patients. Antimicrob. Agents Chemother. 49, 650-655.

SINGH, R., R. K. CHAUDHARY, V. K. DUMKA (2008): Influence of paracetamol on the pharmacokinetics and dosage regimen of ceftizoxime in cross bred calves. Israel J. Vet. Med. 63, 72-76.

STEINER, I. M., H. LANGENBERGER, C. MARSIK, B. X. MAYER, M. FISCHER, A. GEORGOPOULOS, M. MÜLLER, G. HEINZ, C. JOUKHADAR (2004): Effect of norepinephrine on cefpirome tissue concentrations in healthy subjects. J. Antimicrob. Chemother. 53, 506-511.

SULTANA, N., M. S. ARAYNE (2007): *In vitro* activity of cefadroxil, cephalexin, cefatrizine and cefpirome in presence of essential and trace elements. Pak. J. Pharm. Sci. 20, 305-310.

Received: 9 December 2010
Accepted: 6 May 2011

RAJPUT, N., V. K. DUMKA, H. S. SANDHU: Kinetika raspodjele cefpiroma i njegovo *in vitro* vezanje na proteine plazme u goveda. Vet. arhiv 82, 1-9, 2012.

SAŽETAK

Istražena je raspodjela cefpiroma i njegovo *in vitro* vezanje na proteine plazme u petero muške bivolje teladi nakon jednokratne intramuskularne primjene u dozi od 10 mg/kg. Koncentracija cefpiroma u plazmi bila je procijenjena pomoću mikrobioloških testova. Njegovo vezanje na proteine plazme određeno je za različite koncentracije pomoću dijalize. Vršna razina cefpiroma u plazmi nakon intramuskularne primjene postignuta je 45 minuta nakon davanja, a lijek je u plazmi bio dokazan iznad MIC od 0,5 μg/mL do 10 sati nakon davanja. Raspodjela lijeka bila je sukladna modelu otvorenosti jednog odjeljka. Vrijednost $t_{1/2Ka}$ iznosila je 0,21 ± 0,01 h, $t_{1/2\beta}$ 2,06 ± 0,02 h, a AUC 31,7 ± 0,95 μg/mL/h. Cefpirom se vezao na proteine plazme u visini od 26,0 ± 2,84 % u razmaku koncentracije od 1-100 μg/mL. Sposobnost vezanja cefpiroma na proteine plazme bila je 3,71 $\times 10^{-8}$ ± 0,31 $\times 10^{-8}$ mol/g, a konstanta njegova oslobađanja od kompleksa protein-lijek iznosila je 3,43 $\times 10^{-7}$± 0,46 $\times 10^{-7}$ mola.

Ključne riječi: telad, cefpirom, raspodjela, vezanje na proteine