Iranian Journal of Veterinary Research, Shiraz University, Vol. 10, No. 1, Ser. No. 26, 2009

Pharmacokinetics of ceftriaxone in buffalo calves (*Bubalus bubalis*) following intravenous and intramuscular administration

Gohil, P. V.; Patel, U. D.; Bhavsar, S. K.* and Thaker, A. M.

Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388001, Gujarat State, India

***Correspondence:** S. K. Bhavsar, Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388001, Gujarat state, India. E-mail: skbhavsar@aau.in

(Received 6 Apr 2008; revised version 21 Sept 2008; accepted 5 Oct 2008)

Summary

Pharmacokinetics of ceftriaxone was studied in buffalo calves (*Bubalus bubalis*) after single intravenous and intramuscular administration of 10 mg/kg body weight. The drug concentrations in plasma samples were measured by high performance liquid chromatography with UV detection. Following intravenous administration, the drug was rapidly distributed (Cp^o: 106.5 ± 9.64 μg/ml; $t_{1/2\alpha}$: 0.09 ± 0.01 h; Vd_{area}: 0.48 ± 0.05 L/kg) and eliminated ($t_{1/2\beta}$: 1.27 ± 0.04 h) from the body with a clearance rate of 4.40 ± 0.44 ml/min.kg. Following intramuscular administration, the peak plasma concentration of the drug was 15.8 ± 2.4 μg/ml at 0.5 h and the drug was detected up to 12 h. The drug was rapidly absorbed from the site of injection ($t_{1/2ka}$: 0.35 ± 0.01 h), widely distributed (Vd_{area}: 1.53 ± 0.2 L/kg) and slowly eliminated from the body ($t_{1/2\beta}$: 4.38 ± 0.4 h; Cl_B: 4.01 ± 0.30 ml/min.kg). The bioavailability of ceftriaxone was 70.2 ± 2.0% following intramuscular injection. Intramuscular injection of ceftriaxone has favourable pharmacokinetics and moderate bioavailability in buffalo calves and can be used for susceptible infections in calves.

Key words: Pharmacokinetics, Ceftriaxone, Buffalo calves, Intravenous, Intramuscular

Introduction

Ceftriaxone is a third-generation semisynthetic bactericidal cephalosporin, resistant to various types of bacterial β-lactamases. It is effective against a wide variety of Gram-positive and Gram-negative bacteria including Enterobacteriaceae, *Haemophilus influenzae*, *Streptococcus pneumoniae* and methicillin-susceptible staphylococci (Brogden and Ward, 1988; Matsuzaki *et al*., 2005). It has rapid absorption and wide distribution in tissues as well as body fluids, with good tolerance after parenteral administration in animals. The drug thus seems to be extremely useful in a variety of infections including meningitis, septicemia, pyoderma, colibacillosis, surgical prophylaxis and in urinary tract, respiratory tract, wound, soft tissues and joints infections.

Pharmacokinetics of ceftriaxone has been investigated in cow calves, goats, sheep and camel (Soback and Ziv, 1988; Johal and Srivastava, 1998, 1999; Maradiya, 2004; Ismail, 2005; Goudah *et al*., 2006; Goudah, 2008). Despite the great potential for clinical use of this drug in veterinary medicine, data on its pharmacokinetics in buffalo calves are limited except the study done by Dardi *et al*. (2004) concerning intravenous admin-istration of ceftriaxone in buffalo calves. Pharmacokinetic data of ceftriaxone after intramuscular administration are lacking in buffalo calves. Therefore, the present study was planned to determine the pharmacokinetics of ceftriaxone in buffalo calves following single intravenous and intramuscular administration at the dose of 10 mg/kg body weight.

Materials and Methods

Experimental animals and drug administration

Six healthy male buffalo calves of 9-10 months of age, weighing between 70-100 kg were used in the present study. The animals were kept under constant observation for two weeks before commencement of the experiment. They were subjected to routine clinical examination during this period in order to exclude possibility of any disease. They were housed in sheds with concrete floor and were maintained on concentrate, green fodder and dry grass. Water was provided *ad libitum.* Standard and uniform management practices were followed so that the buffalo calves remained free from stress and diseases. Calves were randomly allocated to receive either intravenous or intramuscular injection of ceftriaxone sodium (Vetaceph, Unichem Pharmaceuticals Ltd., India) at the dose of 10 mg/kg. A washout period of 3 weeks was considered between treatments.

Collection of samples

Blood samples (5 ml each) were collected through the intravenous catheter fixed in contralateral jugular vein into heparinized glass test tubes before administration and at 2, 5, 10, 15, 30 min and 1, 1.5, 2, 4, 6, 8, 12, 24, and 36 h after intravenous and intramuscular administration of the drug. Plasma was separated by centrifugation at 3,000 rpm for 10 min at room temperature and stored at -20°C until analysis, which usually took place within 24 h of collection.

Bioassay of ceftriaxone and pharmacokinetic analysis

Plasma ceftriaxone concentration was determined by the high performance liquid chromatography (HPLC) with minor modifications (Hakim *et al*., 1988). The HPLC system (Merck-Hitachi LaChrom) consists of isocratic pump (L-7110) with an online degasser (L-7612), interface (D-7000), UV detector (7400), autosampler (7200), sample cooler (L-7200), chromatography data station software (D-7000) and multi HSM-manager. Chromatographic separation was done using Lichrocart RP-18 column (250 mm × 4 mm) at room temperature.

Samples (250 µl) were deproteinized by addition of acetonitrile (500 µl), vortexed for one min followed by centrifugation for 10 min at 5,000 rpm. A clear supernatant fluid was decanted in a glass insert (automatic sampler vessels) from which 50 µl was injected into the HPLC system. The mobile phase consisted of a mixture of buffer and acetonitrile (62:38). The buffer was prepared by dissolving 1.78 g of di-sodium hydrogen phosphate dihydrate and 1.0 g of N-acetyl-N, N, N-trimethyl ammonium bromide in 950 ml of Milli-Q water, pH (7.0) was adjusted with orthophosphoric acid. Mobile phase was filtered through 0.45 µm Millipore filter. Mobile phase was pumped through column at a flow rate of 1.0 ml/min, at an ambient temperature of 25°C. The elute was monitored at a wavelength of 254 nm. All chemicals used in the present study were of HPLC grade.

Ceftriaxone standards (0.19, 0.26, 0.52, 1.68, 4.93, 14.94, 49.79, 76.59, 90.11, 100.12 µg/ml) were prepared by serial dilutions of stock solution (100.12 µg/ml) in drug-free plasma of calves. Calibration curve was prepared for drug concentrations ranging from 0.19 to 100.12 µg/ml and was used to quantify the drug concentration in samples. The calibration curve was prepared daily and not accepted unless it had a R^2 value ≥0.99. The assay was linear for drug concentrations of 0.19 to 100.12 µg/ml ($R^2 \geq 0.99$). The lower limit of quantification of assay was 0.19 µg/ml. Different pharmacokinetic parameters were calculated by least square linear regression technique described by Gibaldi and Perrier (1982). Formulas used to compute various pharmacokinetic parameters are as follows:

a) Half-life: distribution, elimination and absorption phases:

i) $t_{1/2\alpha} = \frac{0.693}{\alpha}$

ii) $t_{1/2\beta} = \frac{0.693}{\beta}$

iii) $t_{1/2k(a)} = \frac{0.693}{k_{(a)}}$

b) $AUC_{(0-\infty)}$, the total area under the serum drug concentration–time curve and AUMC, the area under the first moment of the serum drug concentration–time curve were calculated by trapezoidal rule.

c) $Vd_{(area)}$, the apparent volume of distribution:

$$Vd_{(area)} = \frac{\text{Dose (mg/kg)}}{\beta \times (AUC)}$$

d) $Vd_{(ss)}$, the volume of distribution of drug at steady state:

$$Vd_{(ss)} = \frac{\text{Dose} \times AUMC}{(AUC)^2}$$

e) Cl_B, the total body clearance of drug:

$$Cl_B = \beta \times Vd_{(ss)} \times 1000$$

f) MRT, the mean residence time:

$$MRT = \frac{AUMC}{AUC}$$

g) F, the fraction of drug absorbed after non-vascular administration:

$$F = \frac{t_{1/2\beta}\ (I.V.) \times AUC\ (I.M.)}{t_{1/2\beta}\ (I.M.) \times AUC\ (I.V.)}$$

Results

Following intravenous and intramuscular administration, experimental data were best fitted to two-compartment ($Cp = Ae^{-\alpha t} + Be^{-\beta t}$) and one-compartment open model ($Cp = Be^{-\beta t} - Ae^{k(a)t}$), respectively. Where Cp is the plasma concentration at time t; A, B and A' are the zero time intercept for distribution, elimination and absorption phases, respectively. α, β and k(a) are the first order rate constant related to distribution, elimination and absorption phases, respectively and e is the base of natural logarithm. The drug was detected in plasma up to 8 and 12 h following intravenous and intramuscular administration, respectively. Comparative disposition of ceftriaxone following single intravenous and intramuscular administration in buffalo calves was plotted on semilogarithmic scale (Fig. 1).

Following intravenous administration of the drug, values of elimination half-life ($t_{1/2\beta}$), total body clearance (Cl_B), apparent volume of distribution ($Vd_{(area)}$) and area under the curve (AUC) were 1.27 ± 0.04 h, 4.40 ± 0.44 ml/min.kg, 0.48 ± 0.05 L/kg and 40.0 ± 4.27 μg.h/ml, respectively. Following intramuscular administration, peak plasma concentration of 15.8 ± 2.4 μg/ml was observed at 0.5 h. The values of elimination half-life ($t_{1/2\beta}$), total body clearance (Cl_B), apparent volume of distribution ($Vd_{(area)}$) and area under the curve (AUC) were 4.38 ± 0.40 h, 4.01 ± 0.30 ml/min.kg, 1.53 ± 0.20 L/kg and 29.7 ± 2.2 μg.h/ml, respectively. Bioavailability of the drug following intramuscular injection was calculated to be 70%. Various pharmacokinetic parameters calculated from plasma concentration of ceftriaxone after its single intravenous and intramuscular administrations are summarized in Table 1.

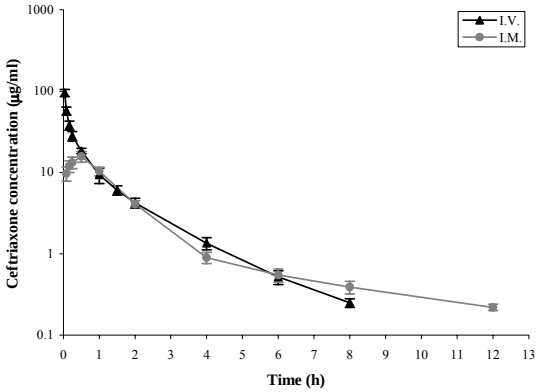

Fig. 1: Semilogarithmic plot of ceftriaxone concentration in plasma versus time following single intravenous (▲) and intramuscular (●) administration at the dose of 10 mg/kg of body weight in buffalo calves. Each point represents mean ± SE of six calves

Discussion

Following intravenous administration, the drug was distributed fast as evidenced by short distribution half-life. This fast distribution of ceftriaxone after intravenous administration in buffalo calves is in agreement with the finding obtained by Dardi *et al*. (2004). Ceftriaxone was rapidly excreted from the body of buffalo calves. The elimination half-life and clearance of ceftriaxone following intravenous administration attained in the present study are in accordance with the half-life (1.39-1.58 h) and clearance (3.35-3.39 ml/min.kg)

Table 1: Pharmacokinetic parameters of ceftriaxone in buffalo calves after single intravenous and intramuscular administration (10 mg/kg of body weight)

Parameter	Unit	Intravenous (Mean ± SE, n = 6)	Intramuscular (Mean ± SE, n = 6)
$t_{1/2\alpha}$	h	0.09 ± 0.01	-------
$t_{1/2\beta}$	h	1.27 ± 0.04	4.38 ± 0.4
$t_{1/2\ k(a)}$	h	-------	0.35 ± 0.01
AUC	μg.h/ml	40.0 ± 4.27	29.67 ± 2.2
AUMC	μg.h^2/ml	53.62 ± 6.24	77.45 ± 6.7
$Vd_{(area)}$	L/kg	0.48 ± 0.05	1.53 ± 0.2
$Vd_{(ss)}$	L/kg	0.36 ± 0.04	0.92 ± 0.1
Cl_B	ml/min.kg	4.40 ± 0.44	4.01 ± 0.3
MRT	h	1.34 ± 0.05	2.63 ± 0.2
F	%	-------	70.2 ± 2.0
C_{max}	μg/ml	-------	15.8 ± 2.4
T_{max}	h	-------	0.5

$t_{1/2\alpha}$: half-life of distribution phases; $t_{1/2\beta}$: elimination half-life; $t_{1/2k(a)}$: absorption half-life; AUC: total area under plasma drug concentration-time curve; AUMC: area under moment curve; $Vd_{(area)}$: apparent volume of distribution; $Vd_{(ss)}$: volume of distribution at steady state; $Cl_{(B)}$: total plasma clearance; MRT: mean residence time; F: bioavailability; C_{max}: maximum drug concentration; T_{max}: time of maximum observed concentration in plasma. -------: parameter has non-significant importance for route of administration

observed in cow calves (Soback and Ziv, 1988; Maradiya, 2004). Similar to our findings, short elimination half-life of 1.75 ± 0.02 h in sheep (Goudah *et al*., 2006), 1.44 h in goats (Ismail, 2005) and 2.57 ± 0.52 h in camels (Goudah, 2008) has been reported. On the contrary, long elimination half-life (4.39 ± 0.63 h) and fast clearance (5.16 ± 0.16 ml/min.kg) of the drug has also been recorded in cow calves (Johal and Srivastava, 1999). The drug was moderately distributed following intravenous administration in buffalo calves as evidenced by $Vd_{(area)}$. Apparent volume of distribution of 0.44 ± 0.07 L/kg for ceftriaxone reported in cow calves (Maradiya, 2004) also supports our observation.

Following intramuscular administration of ceftriaxone in buffalo calves, the peak plasma concentration (15.8 ± 2.4 μg/ml) was achieved at 0.5 h. It is in agreement with the peak plasma concentration of 15.3 ± 2.4 μg/ml observed in cow calves (Maradiya, 2004). Short absorption half-life of ceftriaxone showed that the drug absorbed faster from the injection site. Rapid absorption following intramuscular injection has also been reported for cefotaxime (Sharma *et al*., 2004) and cefoperazone (Goyal *et al*., 2005) in buffalo calves. In the present study, the elimination of ceftriaxone was rapid following intramuscular administration in buffalo calves. This observation is in agreement with the elimination half-life (5.02 ± 0.51 h) of ceftriaxone observed in cow calves (Maradiya, 2004). However, short elimination half-life of the drug has been reported in goats, sheep and camels (Ismail, 2005; Goudah *et al*., 2006; Goudah, 2008). This was further supported by fast clearance (4.40 ± 0.44 ml/min.kg) of the drug in buffalo calves in the present study. Fast clearance (5.66 ± 0.33 ml/min.kg) of the drug has also been reported in cow calves (Johal and Srivastava, 1998). Rapid elimination of cefotaxime (Sharma *et al*., 2004) and cefoperazone (Goyal *et al*., 2005) following intramuscular administration in buffalo calves justifies the observations made in the present study.

The drug is extensively distributed following intramuscular injection as evidenced by high $Vd_{(area)}$ (1.53 ± 0.2 L/kg) and $Vd_{(ss)}$ (0.92 ± 0.1 L/kg). High apparent volume of distribution of ceftriaxone (1.40 ± 0.07 L/kg; Dardi *et al*., 2004) and cefotaxime (1.30 L/kg; Sharma *et al*., 2004) in buffalo calves supports our results. However, moderate distribution of the drug ($Vd_{(area)}$: 0.55 L/kg) has also been reported following intramuscular administration of ceftriaxone in cow calves (Soback and Ziv, 1988).

In the present study, bioavailability of

the drug following intramuscular administration is found in agreement with the bioavailability (78%) obtained in cow calves (Soback and Ziv, 1988). It is further supported by high bioavailability (86.7%) of the drug following intramuscular injection in buffalo calves (Dardi *et al*., 2004). However, lower bioavailability (40%) of ceftriaxone has also been reported in cow calves (Johal and Srivastava, 1998). The pharmacokinetic characteristics of ceftriaxone in buffalo calves following intravenous and intramuscular administration indicates favourable pharmacokinetic profile, therefore, the drug may be used to treat susceptible bacterial infections in buffalo calves.

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