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Subcutaneous pharmacokinetics of ceftazidime in buffalo calves

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ABSTRACT

The pharmacokinetics and urinary excretion of ceftazidime, a third generation cephalosporin, was investigated in buffalo calves (6) following a single subcutaneous administration (10 mg/kg). Ceftazidime concentrations in plasma and urine were estimated by microbiological assay technique using *Escherichia coli* as test organism. Pharmacokinetic analysis of disposition data indicated that subcutaneous administration data were best described by 1-compartment open model. The peak plasma levels of ceftazidime were 24.1±0.26 µg/ml at 45 min and the drug was detected upto 14 h. The absorption half-life and elimination half-life were 0.38±0.07 h and 3.32±0.23 h, respectively. The apparent volume of distribution and total body clearance were 0.18±0.01 L/kg and 39.2±1.22 ml/kg/h, respectively. The urinary excretion of ceftazidime in 36 h, was 29.9±5.34% of total administrated dose. An efficacy predictor, measured as the time over which the active drug exceeds the bacterial minimum inhibitory concentration (T > MIC), was calculated. T > MIC was in the range 91–137% of the recommended dosing interval (8–12 h) after subcutaneous administration, for bacteria with a MIC_{90} ≤.25 µg/ml.

Key words: Buffalo Calves, Ceftazidime, Pharmacokinetics, Urinary excretion

Ceftazidime, an aminothiazolyl third generation cephalosporin antimicrobial agent, is bactericidal and acts by binding to penicillin-binding proteins of gram-negative bacteria to inhibit the cross linking of bacterial peptidoglycan, thereby interfering with bacterial cell wall synthesis (Klein and Cunha 1995). It is active against some susceptible gram-negative bacilli (*Escherichia coli, Proteus* spp.*, Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp.*)* and gram-positive pathogens (*Staphylococcus* spp., *Streptococcus* spp.) and, is very active against *Pseudomonas aeruginosa* (Albarellos *et al*. 2008). Its pharmacokinetic parameters vary widely requiring different dosage regimens and adjustment methods for each agent. There is limited data on the pharmacokinetics of ceftazidime in animals. Ceftazidime was studied in mice (Kita *et al.*1992), rats (Matsui *et al.*1984, Kita *et al.*1992), rabbits (Carbon *et al.* 1984, Sakata *et al.* 1984, Kita *et al.* 1992 Abd-El-Aty *et al.* 2001), monkeys (Matsui *et al.* 1984, Kita *et al.* 1992), calves (Soback and Ziv 1989), sheep (Rule *et al.* 1991), cows (Rule *et al.* 1996), dogs (Matsui *et al.* 1984, Kita *et al.* 1992, Moore *et al.* 2000) and cats (Albarellos *et al.* 2008). However, to our knowledge, pharmacokinetic studies in buffalo calves have not been reported. As the usefulness of an antibacterial agent depends on its efficacy, safety and pharmacokinetic disposition in the target animal, the aim of present study was to investigate the pharmacokinetics and urinary excretion of ceftazidime following single subcutaneous administration in buffalo calves. In veterinary practice, administration of antibiotics by subcutaneous route was found very effective (Sharma and Pathania 2010).

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MATERIALS AND METHODS

Animals: Healthy male buffalo calves (6), 6–12 months of age and weighing between 85 and125 kg body weights were used. The animals were housed in an animal shed with concrete floor and adequate ventilation. The animals were determined to be clinically healthy before the study. All the animals were acclimatized in the animal shed under uniform conditions and were maintained on green fodder, wheat straw and water *ad lib.* They did not receive any drug treatment before the study. For the collection of urine, the experimental animals were kept in metabolic stalls of standard size, 12h before the start of experiment and kept there for entire study. The metabolic stalls are designed in such a way that urine voided by animals can be collected at any time interval without any spillage.

Experimental design

Aqueous solutions of ceftazidime-pentahydrate were administered by subcutaneous route @ 10 mg/kg as 10% solution. Blood samples (4–6 ml) were taken from contra

lateral jugular vein into heparinized glass test tubes before administration and at different time intervals *viz.* 2.5, 5, 10, 15, 30, 45 min and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14 h in each group. Plasma from the samples was separated by centrifugation at 2000 rpm for 15 min and stored at –20°C till analysis usually the next day. Urine was collected at 4, 8, 12, 16, 20, 24, 28, 32, 36 h after administration of drug. The urine voided by animals was filtered and measured and approximately 5 ml urine sample was stored at –20°C till analysis.

Bioassay: The concentrations of ceftazidime in plasma and urine were estimated by microbiological assay technique (Arret *et al.* 1971) using *Escherichia coli* (MTCC 739) as the test organism. The bioassay method used in this work could not distinguish between the parent compound and its active metabolites, if they exist. However, it measured the overall microbiological activity of the drug. The standard curve of ceftazidime in buffalo calf plasma was linear between 1 to 5 µg/ml. The value of coefficient of determination (r^2) of the standard curve was 0.99. The drug could be detected up to a minimum limit of 1 µg/ml. The ceftazidime recovery exceeded 96% from plasma and urine over the concentration range of 5 to 200 µg/ml. The intra-day and inter-day coefficients of variance were less than 3%.

Pharmacokinetic analysis: The plasma concentration time data for each buffalo calf were determined according to the computed least squares regression technique. One compartment open system was found to best fit the data, following subcutaneous administration. The kinetic parameters were calculated from the formulae derived for a single compartment open model (Gibaldi and Perrier 1982).

RESULTS AND DISCUSSION

Clinical examination of all animals before and after each trial did not reveal any abnormalities. No adverse reactions were observed after administration of ceftazidime in the animals studied. The mean plasma concentration–time profiles of ceftazidime following single SC administration are presented in Fig.1. Evaluation of the results on observed plasma levels of ceftazidime indicated that the data can be best fitted to one-compartment open model with the exponential equation:

$$Cp = Be^{-ßt} - A^1e^{-Kat}$$

where, Cp is the ceftazidime concentration at time t, A^1 and B are zero-time intercepts of absorption and elimination phases of the plasma concentration-time curves, respectively, Ka and ß are the absorption and elimination rate constants, respectively, and e represents the base of natural logarithms.

Similar to our study ceftazidime has been reported to follow mono-compartment open model in unweaned calves (Soback and Ziv 1989), lactating and non lactating cows (Rule *et al.* 1996), dogs (Moore *et al.* 2000) and cats (Albarellos *et al.* 2008) after extravascular administration.

The plasma concentration-vs-time curve after SC administration documented C_{max} (24.1±0.26 µg/ml) at 0.75 h, indicating fast absorption. The minimum therapeutic plasma concentration (0.25 µg/ml) was maintained from 2.5 to 14 h. The rapid appearance of ceftazidime in the plasma suggests that this drug quickly enters into the systemic circulation following subcutaneous administration. Mean

Table 1. Pharmacokinetic parameters of ceftazidime in buffalo calves after a single subcutaneous injection (10 mg/kg)

Parameter	Unit	Mean±SE
A	µg/ml	35.4±3.90
K_a	h^{-1}	2.26±0.53
$t_{½Ka}$	h	0.38±0.07
B	µg/ml	35.9±4.08
β	h^{-1}	0.21±0.02
$t_{½β}$	h	3.32±0.23
AUC	µg/ml.h	141.0±4.93
AUMC	µg/ml.h^2	792.5±61.7
$V_{d(area)}$	L/kg	0.18±0.01
$V_{d(B)}$	L/kg	0.30±0.05
Cl_B	ml/kg/h	39.2±1.22
MRT	h	5.59±0.29
C_{max}	µg/ml	24.1±0.26
t_{max}	h	0.75±0

Kinetics parameters are as described by Gibaldi and Perrier (1982). A and B, Zero-time plasma drug concentration intercept of the regression line ofabsorption and elimination phases, respectively; K_a and ß are the absorption and elimination rate constants, respectively; $t_{½Ka}$, absorption half-life; $t_{½ß}$, elimination half-life; AUC, area under the plasma concentration-time curve; AUMC, area under the first-moment curve; $Vd_{(area)}$, apparent volume of distribution based on AUC; $Vd_{(B)}$, volume of distribution based on zero-time plasma drug concentration intercept of elimination phase; Cl_B, total body clearance; MRT, mean residence time; C_{max}, the peak or maximum plasma concentration; $t_{max,}$ the time to reach peak or maximum plasma concentration.

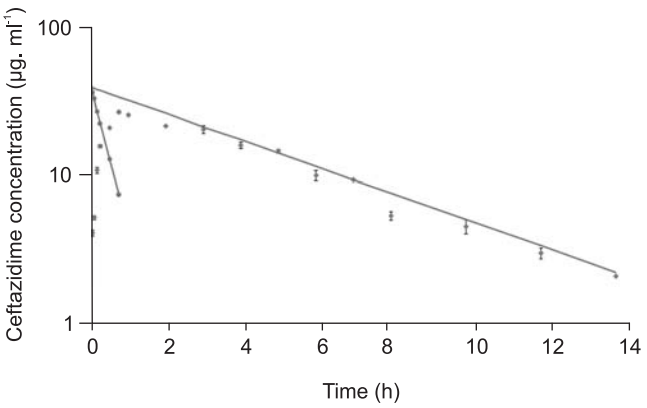

Fig 1. Semi-logarithmic plot of plasma concentration-time profile of ceftazidime in buffalo calves following a single subcutaneous dose of 10 mg/kg body weight.

Values given are mean±SE of 6 animals. The calculated points (●) of distribution phase are calculated by the feathering technique.

(±SE) values for pharmacokinetic parameters are given in Table 1. The elimination half life ($t_{1/2ß}$) of ceftazidime in buffalo calves was longer than reported in unweaned calves (Soback and Ziv 1989), sheep (Rule *et al.* 1991), dogs and mice (Kita *et al.* 1992), lactating and non-lactating cows (Rule *et al.* 1996), rabbits (Abd El Aty *et al.* 2001) and cats (Albarellos *et al.* 2008).

The relatively low volume of distribution in buffalo calves (0.18±0.01 L/kg) was the expected for a beta lactam antibiotic. This value was consistent with that reported in dogs (Matsui *et al.* 1984) and cats (Albarellos *et al.* 2008), but, was smaller than reported in unweaned calves (Soback and Ziv 1989), sheep (Rule *et al.* 1991), lactating and non-lactating cows (Rule *et al.* 1996). The value of volume of distribution in dogs, cats, unweaned calves, sheep, lactating and non-lactating cows was 0.21±0.0007, 0.18±0.04, 0.29±0.06, 0.35±0.21, 0.49±0.14 and 0.39±0.21 L/kg, respectively. The total body clearance (Cl_B) of ceftazidime 39.2 ± 1.22 ml/kg/h in buffalo calves recorded in this study was smaller than dogs (Matsui *et al.* 1984), unweaned calves (Soback and Ziv 1989), lactating and non-lactating cows (Rule *et al.* 1996) and cats (Albarellos *et al.* 2008). The value of Cl_B reported in dogs, unweaned calves, lactating and non-lactating cows and cats was 215±3,105±15.6, 72.5±18.1, 185.9±44.2 and 190±80 ml/kg/h,respectively. Species differences are relatively common and are frequently related to inter-species variation, assay method used, the amount of time between blood samplings, the health status, the age of the animal, dosing, frequency of administration, dose extrapolation etc.

The urinary excretion of ceftazidime in buffalo calves is presented in Table 2. Ceftazidime is mostly eliminated by glomerular filtration (Soback and Ziv 1989; Verhagen *et al.* 1994, Albarellos *et al.* 2008). The cumulative percent of total dose excreted in urine, after SC administration was 29.9±5.34% within 36 h. In contrast to our findings the cumulative per cent of ceftazidime excreted in urine of rats and dogs (Matsui *et al.* 1984) and mice (Kita *et al.* 1992) was 97.1, 86.3 and 77.9%, respectively. The peak urine level of drug (497.4±122.8 µg/ml) was detected at 4 h and there after the level remain ≥8µg/ml in urine up to 24 h of administration. The concentration of ceftazidime in urine of buffalo calves remained higher than the MIC (0.25 to 8 µg/ml) of most microorganisms (Soback and Ziv 1989,Moore *et al*. 2000, Casellas *et al*. 2003, Rhomberg *et al*. 2004,Albarellos *et al.* 2008) sensitive to the drug up to 24h. This suggested that use of ceftazidime in buffalo calves might achieve successful bacterial killing in urinary tract infection caused by microorganisms having susceptibility ≤8 µg/ml.

Table 2. Urinary excretion of ceftazidime in buffalo calves after a single subcutaneous injection (10 mg/kg)

Time interval (h)	Concentration (µg/ml)	Total (%) dose excreted
0–4	497.4±122.8	18.1±1.77
4–8	348.9±88.7	9.30±2.29
8–12	200.5±49.3	4.74±1.19
12–16	53.6±29.0	1.01±0.52
16–20	28.4±7.62	0.87±0.33
20–24	8.06±6.23	0.09±0.03
24–28	6.21±1.91	0.12±0.07
28–32	4.05±0.26	0.10±0.04
32–36*	3.44	0.18
0–36	-	29.9±5.34

The values given are mean±SE of the results obtained from 4–6 animals.*Values obtained from 2 animals.

The success of antimicrobial therapy is determined by complex interactions between an administered drug, a host, and an infecting agent. In a clinical situation, the complexity of these interactions is usually reflected by a high variability in the dose-response relationship. Therefore, to minimize the dose-response variability, key characteristics of the drug, the infecting agent and the host have to be taken into account for selecting an appropriate antibiotic and an appropriate dose. Failure to do so may result in either therapeutic failure or emergence of resistant strains. In recent years substantial efforts were devoted to systematically elucidate the dynamic relationship between pharmacokinetic and pharmacodynamic variables. The main concept of this pharmacokinetic-pharmacodynamic approach is to use the concentration-effect relationship of the drug of interest in dosage adjustment and product development in a logical way and minimize trial-and-error approaches (Derendorf 1989, Meibohm and Derendorf 1997).

For ß-lactam antibiotics, the most important pharmacokinetic- pharmacodynamic index correlating with *in vivo* efficacy has been shown to be the duration that the unbound concentration of an antibiotic remains above the MIC as a percentage of the dosing interval (%T>MIC) (Craig 1998, Andes and Craig 2002). Their effect will increase with increasing concentrations until a finite point (the maximum kill rate) is reached. After that point, increasing concentrations will not produce a corresponding increase in the effect; therefore, high peak concentration will not help. For cephalosporins, a T > MIC 35–40% of the interdose interval has been established as optimal for bacteriostatic action, while a T > MIC of 60–70% is necessary for a bactericidal effect (Craig 1998, Toutain *et al.*2002). The calculated T> MIC (%) after SC ceftazidime administrations and for two dosing intervals (8 and 12 h) are presented in

Table 3. Time above ceftazidime minimum inhibitory concentration (T > MIC) expressed as percentage of the inter-dose interval (8 or 12 h) for the subcutaneous (SC) administration to buffalo calves (10 mg/kg)

Inter-dose	MIC (µg/ml)		
interval (h)	0.25	4.0	8.0
8	136.8	57.2	37.3
12	91.2	38.1	24.9

Table 3. In this study, subcutaneous ceftazidime administrations at dose of 10 mg/kg seem to be suitable for the inter-dose interval proposed (12 h) against bacterial isolates with MIC ≤ 0.25µg/ml. Furthermore, clinical controlled trials are mandatory to establish proper ceftazidime dosing schedules in this species.

In conclusion, on the basis of the results reported above, ceftazidime (10 mg/kg) shows favourable pharmacokinetic behaviour, but needs to be evaluated for clinical efficacy and safety in disease conditions in buffalo species before issuing final recommendations. It would appear to be a good therapeutic tool for the treatment of most of the infections produced by gram-negative and gram-positive susceptible bacteria in buffalo calves.

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