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Effect of *E. coli* lipopolysaccharides-induced fever on the disposition pattern of cefotaxime in buffalo calves

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ABSTRACT

The pharmacokinetics, urinary excretion and dosage regimen of cefotaxime after its single intravenous administration (10 mg/kg) was investigated in experimentally induced fever in buffalo calves (n = 5). The fever was induced by intravenous injection of *E. coli* lipopolysaccaride (1 μg/kg). At 1 min. the concentration of cefotaxime in plasma was 90.9 ± 2.85 μg/mL, which rapidly declined to 26.2 ± 1.05 μg/mL at 10 min. The drug was detected for up to 8 h. The elimination half-life and volume of distribution were 1.85 ± 0.11 hr and 1.70 ± 0.07 L/kg, respectively. The distribution half-life and area under curve (AUC) were 0.078 ± 0.003 hr and 15.8 ± 0.85 μg/mL.hr, respectively. Total body clearance (Cl_B) and tissue/plasma (T/C) ratio were 644 ± 39.2 mL/kg/h and 15.8 ± 1.04, respectively. Cefotaxime was bound to plasma proteins of febrile buffalo calves to the extent of 32.3 ± 1.86 per cent. To maintain a minimum therapeutic concentration of 1 μg/mL, a satisfactory dosage regimen of cefotaxime in febrile buffalo calves would be 17 mg/kg, followed by 15 mg/kg at 6 h. intervals.

Key words: buffalo calf, cefotaxime, dosage regimen, fever, pharmacokinetics, protein binding, urinary excretion

Introduction

Cefotaxime, a semisynthetic bactericidal cephalosporin, is effective against a wide variety of Gram-positive and Gram-negative microorganisms (NEU et al., 1979). Pharmacokinetics of chemotherapeutic agents are markedly altered in disease conditions (LESAR and ZASKE,

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1984; HARY et al., 1989; TOTH et al., 1991; SINGH et al., 1998; CHAUDHARY et al., 1999; DARDI et al., 2005; SHARMA et al., 2005). Hence, the dosage regimen obtained in healthy subjects cannot be extrapolated in clinical cases to treat diseased animals. Fever, which is one of the most common manifestations of many infectious diseases, is reported to induce a series of biochemical and physiological alterations in cells (VAN MIERT, 1985 and 1987; LOHUIS et al., 1988). Therefore, a study on the influence of fever on the pharmacokinetics of antibiotics is essential. The aim of this study was to determine the disposition kinetic variables of cefotaxime and its urinary excretion in febrile buffalo calves following intravenous administration. From the disposition kinetic data, recommendations were made for optimal dosage regimens of cefotaxime in febrile buffalo calves.

Materials and methods

The experiment was performed on five healthy male buffalo calves of 6-12 months of age, weighing an average of 98 kg. The animals were housed in the departmental shed that had a concrete floor and were provided green fodder and water *ad libitum*. Each animal was quarantined for two weeks before the start of experiment and was determined to be healthy by regular clinical examination. Fever was induced by intravenous administration of *E. coli* lipopolysaccaride at a dose rate of 1 μg/kg b.wt as standardized in our previous study (SHARMA et al., 1996) in buffalo calves. This dose of lipopolysaccaride caused fever within two h., and fever persisted for 4-6 h. At least a 1.1 ^{0}C increase of temperature from normal temperature was taken as the time of cefotaxime administration. Once fever was induced, cefotaxime sodium was injected intravenously to these five animals at a dose rate of 10 mg/kg of cefotaxime, in a 10% solution with sterilized distilled water. Blood samples (5 mL each) were withdrawn from the contralateral jugular vein into heparanized glass test tubes before administration and at 1, 2.5, 5, 7.5, 10, 15, 30, 45, 60, and 90 min. and 2, 3, 4, 5, 6, 7 and 8 h. after administration of drug. Plasma was collected after centrifugation at 2000 g for 15 min. at room temperature and kept at -20 ^{0}C until analysis, usually occurring the next day. Concentration of cefotaxime in plasma was estimated by employing the microbiological assay technique (ARRET et al., 1971) using *Escherichia coli* (ATCC 25922) as the test organism. This method does not distinguish between activity of the parent compound and its metabolites.

The assay could detect a minimum of 0.1 μg/mL of cefotaxime. The standard curve of cefotaxime in calf plasma was linear, between 0.25 and 1.25 μg/mL. The reproducibility of this method was excellent and error within day estimation was less than 5%. Each sample was diluted to the extent that its zone of inhibition came in linear range (preferably in the range of the zone of inhibition of the reference concentration). In this experiment the reference concentration was 0.5 μg/mL. The plasma concentration-time data for each buffalo calf were determined according to the computed least squares regression technique.

Kinetic parameters were calculated from the formulae derived for a two-compartment open model (NOTARI, 1980; GIBALDI and PERRIER, 1982).

The dosage regimen of cefotaxime was also determined based on the kinetic data. The priming (D) and maintenance (D_1) doses are calculated from the equation:

$$D = C_p(\min)^{\alpha}.V_d\, e^{\beta\tau}$$

$$D_1 = C_p(\min)^{\alpha}.V_d(e^{\beta\tau}-1)$$

Results

Table 1. Disposition kinetic parameters of cefotaxime in febrile buffalo calves (n = 5) after a single intravenous injection of 10 mg/kg body mass

Parameter[a]	Unit	Mean ± SE
C^o_p	μg/mL	98.5 ± 3.41
A	μg/mL	96.7 ± 3.44
B	μg/mL	1.84 ± 0.11
α	/h	8.95 ± 0.36
β	/h	0.381 ± 0.026
$t_{1/2\alpha}$	h	0.078 ± 0.003
$t_{1/2\beta}$	h	1.85 ± 0.11
K_{12}	/h	2.48 ± 0.08
K_{21}	/h	0.54 ± 0.03
$K_{12/K21}$ ratio	-	4.65 ± 0.31
AUC	μg/mL.h	15.8 ± 0.85
$V_{d(area)}$	L/kg	1.70 ± 0.07
Cl_B	mL/kg/h	644 ± 39.2
T/P ratio	-	15.8 ± 1.04
t_d	h	10.4 ± 0.63

[a] Kinetic parameters as described by GIBALDI and PERRIER (1982). C^o_p = Plasma drug concentration immediately following intravenous injection of a single dose; A, B = zero-time plasma drug concentration intercepts of regression lines of distribution and elimination phases, respectively; α and β = distribution and elimination rate constants, respectively; $t_{1/2\alpha}$ = distribution half life; $t_{1/2\beta}$ = elimination half life; K_{12}, K_{21} = rate of transfer of drug from central (blood) to peripheral (tissues) compartment and vice-versa; AUC = total area under plasma drug concentration-time curve; $V_{d(area)}$ = apparent volume of distribution; Cl_B = total plasma clearance; T/P = tissue /plasma ratio of drug concentration; t_d = duration of therapeutic plasma concentration.

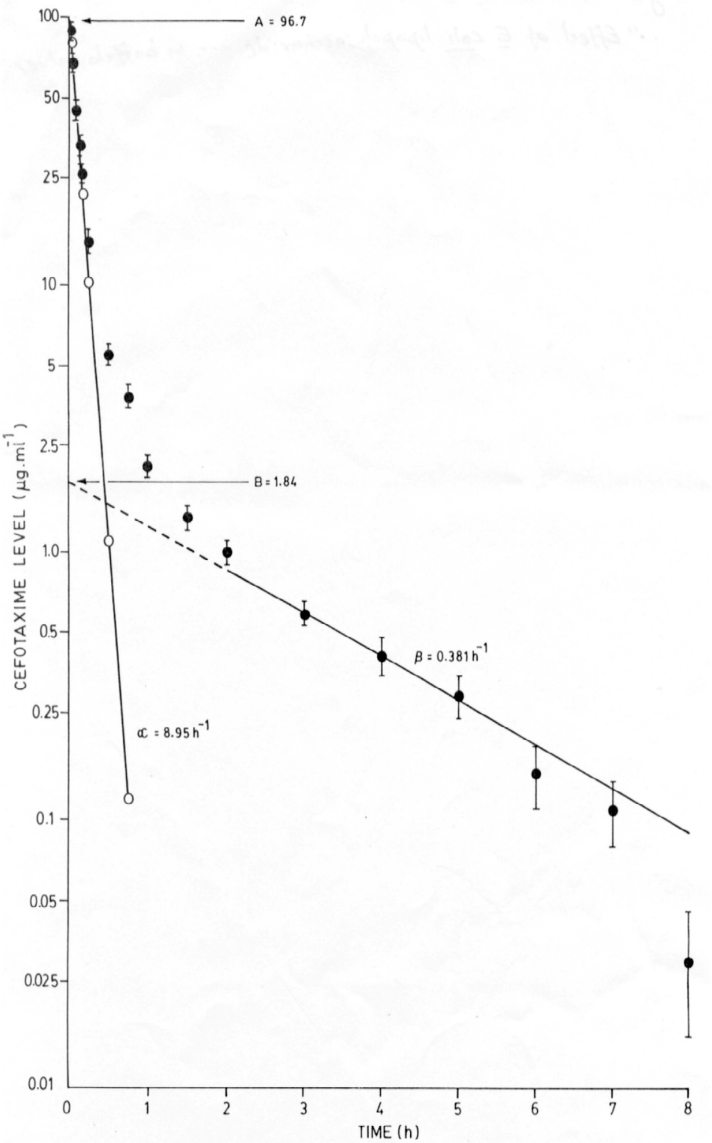

Fig 1. A semi-logarithmic plot of plasma levels of cefotaxime after a single intravenous dose of 10 mg/kg b. wt. in febrile buffalo calves. Values given are mean ± SE of 5 animals. SE shown at 8 h. is one-third of actual SE. Data were analysed according to two-compartment open model. The calculated points (O) of the distribution phase are obtained by feathering techniques.

The mean plasma concentration of cefotaxime as a function of time was plotted on a semilogarithmic scale (Fig.1). At 1 min. the mean plasma concentration of cefotaxime was 90.9 ± 2.85 μg/mL, which rapidly declined to plasma concentration of 26.2 ± 1.05 μg/mL at 10 min. Levels then gradually decreased to 0.03 ± 0.03 μg/mL at 8 h. Various pharmacokinetic parameters for cefotaxime in buffalo calves in which fever was induced before administration of drug are presented in Table 1. Taking 6 and 8h. as convenient dosage intervals (τ) with minimum therapeutic concentration C_p (min)$^{\alpha}$ of 0.2, 0.4, 0.6, 0.8 and 1.0 μg/mL and using the values of β and $V_{d\,(area)}$ of Table 1, the dosage regimen of cefotaxime were computed and are presented in Table 2.

Table 2. Calculated intravenous dosage regimen of cefotaxime required to maintain specified plasma cefotaxime concentration in febrile buffalo calves

Desired plasma concentration (mg/mL)	Dosage interval (hr)	Priming dose (mg/kg)	Maintenance dose (mg/kg)
0.2	6	3.34	3.00
0.2	8	7.16	6.82
0.4	6	6.68	6.00
0.4	8	14.3	13.6
0.6	6	10.0	9.00
0.6	8	21.5	20.5
0.8	6	13.4	12.0
0.8	8	28.6	27.3
1.0	6	16.7	15.0
1.0	8	35.8	34.1

Discussion

Evaluation of results on plasma cefotaxime levels against time indicated that pharmacokinetics of cefotaxime in febrile buffalo calves, after intravenous administration, was best described by the two-compartment open model. The plasma concentration-time data were adequately described by the equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

Where Cp is cefotaxime concentration in plasma at time 't'. A and B are zero time intercepts of the distribution and elimination phase of the plasma concentration time curve. α and β are the distribution and elimination rate constants, respectively, and 'e' represents the base of natural logarithm.

Cefotaxime after intravenous administration has also been reported to follow the two-compartment open model in dogs (GUERRINI et al., 1986), goats (ATEF et al., 1990) and in healthy buffalo calves (SHARMA, 2000). A comparison of plasma levels of cefotaxime in febrile animals with healthy animals (SHARMA, 2000) indicates that the peak plasma levels of cefotaxime in febrile buffalo calves (90.9 ± 2.85 μg/mL) was higher than healthy buffalo calves (71.8 ± 2.49 μg/mL), but generally most of the time the plasma concentration in febrile buffalo calves was similar to healthy animals. The high values of distribution rate constant α (8.95 ± 0.36 /h) indicate that cefotaxime is rapidly distributed into various body fluids and tissue compartments. The rapid distribution of cefotaxime is further substantiated by high values of K_{12}/K_{21} (4.65 ± 0.31). The values of $V_{d(area)}$ of cefotaxime in healthy animals (SHARMA, 2000) is lower (1.17 ± 0.10 L/kg) as compared to febrile animals (1.70 ± 0.07 L/kg) In accordance with our present findings, CHAUDHARY et al. (1999) reported an increase in $V_{d(area)}$ of cefuroxime in febrile buffalo calves as compared to healthy subjects .

While comparing total body clearance in febrile animals with that of healthy animals (SHARMA, 2000), it was found that the value of Cl_B in febrile animals (644 ± 39.2 mL/kg/h) is not significantly different when compared to healthy animals (675.2 ± 38.7 mL/kg/h). Endotoxin causes hepatic, renal dysfunctions (WILKINSON et al. 1974; WILKINSON, 1977) as well as haemodynamic depression (VAN MIERT, 1973). The depressing effect of endotoxin on the renal system could have contributed to the change in volume of distribution in febrile animals. Because of significant alterations in hepatic function the levels of various enzymes, responsible for the metabolism of these antimicrobials, is altered, changing the elimination and biotransformation pattern of the drug during fever (SINGH et al., 1997).

At the end of 24 h., urinary excretion was 4% of total administered dose (Table 2), which was similar to that of healthy buffalo calves (SHARMA, 2000). In healthy crossbred calves, following a single intravenous administration of cefotaxime (10 mg/kg) approximately 4.5% of drug was recovered in urine within 12 h. (SHARMA et al., 1995). TOTH et al. (1991) have also reported that the fraction of the administered dose of ceftriaxone excreted in urine of liver transplant recipients did not differ markedly from that of normal subjects.

The extent of binding of cefotaxime to plasma protein of febrile buffalo calves was 32.3 ± 1.86 per cent. Similarly, cefotaxime was reported to bind plasma proteins of human (NEU, 1982) and crossbred calves (SHARMA and SRIVASTAVA, 1994) to the extent of 36-40 and 30.2 per cent, respectively. However, it may be inferred that the binding of cefotaxime to plasma protein is weak in buffalo calves, which is evident from the low value of β_i and that it is reversible from the high value of the dissociation rate constant for the protein-drug complex.

The ultimate aim of the present study was to determine a satisfactory dosage regimen in febrile buffalo calves. It is not axiomatic to compute the dosage regimen of cefotaxime

to be used effectively in clinical practice for the treatment of mild to severe bacterial infections, without having first conducted a detailed pharmacokinetic study. With a minimum therapeutic plasma concentration of cefotaxime as 1.0 μg/mL (KNUDSEN et al., 1997), which has been shown to be most effective against the majority of sensitive Gram-positive and Gram-negative pathogens, the convenient and suitable dosage regimen of cefotaxime in the febrile buffalo calves after intravenous administration would be 17 mg/kg followed by 15 mg/kg at 6 h. intervals.

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SAŽETAK

Farmakokinetika, izlučivanje mokraćom i režim doziranja cefotaksima nakon njegova jednokratnoga intravenskoga unosa (10 mg/kg) istraženi su u bivolje teladi (n = 5) s pokusno uzrokovanom vrućicom. Vrućica je bila uzrokovana ubrizgavanjem lipopolisaharida bakterije *E. coli* u količini 1 µg/kg. Koncentracija cefotaksima u plazmi nakon jedne minute bila je 90,9 ± 2,85 µg/mL te se brzo spustila na 26,2 ± 1,05 µg/mL u 10. minuti. Lijek se mogao dokazati do osam sati nakon davanja. Poluvrijeme njegova izlučivanja iznosilo je 1,85 ± 0,11 sati, a volumen raspodjele 1,70 ± 0,07 L/kg. Poluvrijeme raspodjele bilo je 0,078 ± 0,003 sati, dok je površina ispod koncentracijske krivulje bila 15,8 ± 0,85 µg/mL/sat. Ukupni tjelesni klirens bio je 644 ± 39,2 mL/kg/sat, a omjer tkivo/plazma bio je 15,8 ± 1,04. Cefotaksim je bio vezan na bjelančevine plazme u febrilne bivolje teladi do 32,3 ± 1,86%. Za održavanje minimalne terapijske koncentracije cefotaksima od 1 µg/mL u febrilne bivolje teladi dostatna je početna doza od 17 mg/kg, a nakon toga slijede doze od 15 mg/kg u razmacima od šest sati.

Ključne riječi: bivolja telad, cefotaksim, doziranje, vrućica, farmakokinetika, vezanje na bjelančevine, izlučivanje u mokraći