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Pharmacokinetics and dosage regimen of ceftriaxone in *E. coli* lipopolysaccharide induced fever in buffalo calves

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The present study was planned to investigate the pharmacokinetics of ceftriaxone in experimentally induced febrile buffalo calves (n = 5). The fever was induced by intravenous injection of *E.coli* lipopolysaccaride (1 μg/kg). To study the pharmacokinetics, ceftriaxone was administered at the dose rate of 10 mg/kg body wt. in all animals. At 1 min, the peak concentration of ceftriaxone was 79.4 ± 2.37 μg/ml and the drug was detected up to 6 h. The elimination rate constant was 0.35 ± 0.02 /h and elimination half-life was 2.04 ± 0.14 h. The apparent volume of distribution ($V_{d(area)}$) and total body clearance (Cl_B) were 1.21 ± 0.15 l/kg and 0.41 ± 0.03 l/kg/h, respectively. To maintain a minimum therapeutic concentration of 1 μg/kg, a satisfactory dosage regimen of cefriaxone in febrile buffalo calves is 19 mg/kg followed by 18 mg/kg at 8 h intervals.

Key words: buffalo calf, ceftriaxone, dosage regimen, febrile, pharmacokinetics.

Introduction

Ceftriaxone is a third-generation semi synthetic bactericidal cephalosporin, which is effective against a wide variety of Gram-positive and Gram-negative microorganisms. The dosage regimen of antibiotics determined in healthy subjects can not be extrapolated to diseased conditions because the disease conditions are reported to markedly alter the pharmacokinetics of several antimicrobial agents [5,9,11, 16,17,20]. Fever, which is one of the most common manifestation of many infectious diseases [8] is reported to induce a series of biochemical and physiological alterations in cells [10,22,23]. So, the study on, influence of fever on the pharmacokinetics of antibiotics is essential. However only meager information is available about the influence of fever on the pharmacokinetics of cephalosporins. [1,5,16,19].

Since there is no information available on the pharmacokinetics and dosage regimen of ceftriaxone in febrile buffalo calves, the present study was therefore planned to calculate the pharmacokinetics of ceftriaxone in febrile buffalo calves. From the pharmacokinetic data, recommendations are made for optimal dosage regimen of ceftriaxone in buffalo calves.

Materials and Methods

The experiment was performed in five healthy male buffalo calves of 10-12 months age and weighing an average weight of 95 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 the dose rate of 1 μg/kg body wt. as standardized in our previous study in buffalo calves [17]. This dose of lipopolysaccaride caused fever with in two hours and fever persisted for 4-6 hours. At least 2°F increase of temperature from the normal temperature was taken as the time of ceftriaxone administration. Once fever was induced ceftriaxone sodium was injected intravenously to these five animals at dose rate of 10 mg/kg of ceftriaxone, in a 10% solution with sterilized distilled water. Blood samples (5 ml each) were withdrawn from the contralateral jugular vein into heparinized glass test tubes before administration and at 1, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, and 90 minutes and 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 h after administration of drug. Plasma was collected after centrifugation at 2000 g for 15 minutes at room temperature and kept at –20°C until analysis, usually the next day. The concentration of ceftriaxone in plasma was estimated by employing the

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microbiological assay technique [3] using *Escherichia coli* (American type cell culture: ATCC 25922) as the test organism.

The assay could detect a minimum of 0.1 μg/ml of ceftriaxone .The standard curve of ceftriaxone in calf plasma was linear between 0.25 and 1.25 μg/ml. The repeatability 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. For the estimation of ceftriaxone, out of six wells on each plate three were filled with reference concentration (0.5 μg/ml) and three wells with diluted sample, and 3 or 4 plates were used for each sample. The pharmacokinetic parameters for ceftriaxone in plasma were calculated using WIN- NONLIN program (SCI software, USA) utilizing non-linear regression. The data gave best fit to the three-compartment model. Akaike information criterion (AIC) and MAICE (minimum Akaike information criterion) values were applied to select the model. The data were re-weighted after selecting the model to obtain better estimates of kinetic parameters.

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

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

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

Results

The mean plasma concentration of ceftriaxone is given in Table 1 and mean plasma concentration as a function of time was plotted on a semilogarithmic scale (Fig. 1). At 1 minute the mean plasma concentration of ceftriaxone was 79.4 ± 2.37 μg/ml, which rapidly declined to plasma concentration of 29.1 ± 4.30 μg/ml at 10 minutes. Then levels gradually decreased to 0.16 ± 0.06 μg/ml at 6 hours. Various pharmacokinetic parameters for ceftriaxone in buffalo calves in which fever was induced before administration of drug are given in Table 2. Taking 6 and 8 h 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 $V_{d(\text{area})}$ of Table 2, the dosage regimen of ceftriaxone were computed and are presented in Table 3.

Discussion

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

$$C_p = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t} + B e^{-\beta\tau}$$

A comparison of plasma levels of ceftriaxone in febrile animals with our earlier study in healthy animals [6], indicates that the peak plasma levels of ceftriaxone in febrile buffalo calves (79.4 ± 2.37 μg/ml) was almost similar to healthy buffalo calves (80.8 ± 5.30 μg/ml), but in general, at most of time, the plasma concentration in febrile buffalo calves was significantly lower than in healthy animals. The marked difference was that in healthy buffalo calves, the plasma level was detected up to 12 h, while in febrile animals it was detected up to 6 h. Accordingly, lower concentration of gentamicin in febrile goats [2] and human beings [12], cefazolin in febrile goats [14] and cefuroxime in

Table 1. Plasma levels of ceftriaxone in febrile buffalo calves after a single intravenous injection of 10 mg/kg /body weight

Time after ceftriaxone administration (min)	Mean±SE (μg/ml)
1	79.4 ± 2.37
2.5	65.3 ± 3.40
5	51.1 ± 2.69
7.5	37.3 ± 3.35
10	29.1 ± 4.30
15	24.9 ± 4.55
20	18.1 ± 3.70
30	11.5 ± 1.86
45	8.13 ± 1.89
60	4.53 ± 0.53
90	2.24 ± 0.36
120	1.01 ± 0.006
180	0.84 ± 0.04
240	0.55 ± 0.04
300	0.38 ± 0.09
360	0.16 ± 0.06

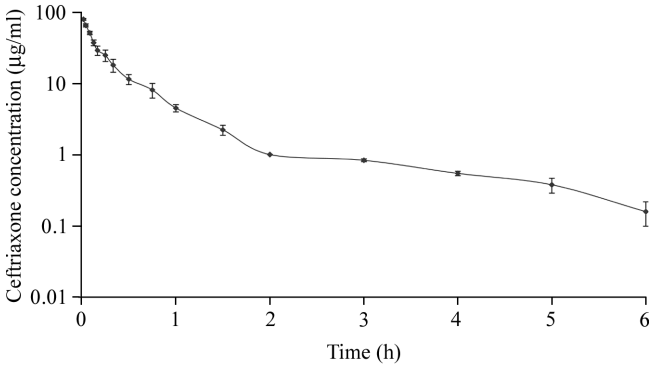

Fig 1. Plasma levels of ceftriaxone after a single intravenous dose of 10 mg/kg (body weight) of buffalo calves, in which fever was induced with intravenous administration of *E. coli* lipopolysaccaride (1 μg/kg). Values given are mean ± SE.

Table 2. Pharmacokinetic parameters of ceftriaxone in febrile buffalo calves after a single intravenous injection of 10 mg/kg (body weight)

Parameter[a]	Mean ± SE (unit)
C^{o}_{p}	93.2±4.79 μg/ml
A_1	57.9±7.90 μg/ml
A_2	33.0±8.26 μg/ml
B	2.24±0.17 μg/ml
α_1	12.0±1.55 /h
α_2	2.36±0.15 /h
β	0.35±0.02 /h
$t_{1/2\alpha1}$	0.06±0.01 h
$t_{1/2\alpha2}$	0.30±0.02 h
$t_{1/2\beta}$	2.04±0.14 h
K_{12}	3.43±0.70 /h
K_{21}	6.08±1.33 /h
K_{12}/K_{21} ratio	0.63±0.14
K_{13}	1.00±0.17 /h
K_{31}	0.45±0.02 /h
K_{13}/K_{31} ratio	2.30±0.48
AUC	25.2±1.97 μg/ml/h
$V_{d(area)}$	1.21±0.15 l/kg
Cl_B	0.41±0.03 l/kg/h
T/P ratio	10.2±1.46
t_d	13.5±0.89 h

[a] Kinetic parameters as described by Gibaldi and Perrier (1982)
C^{o}_{p} = Plasma drug concentration immediately following intravenous injection of single dose; A_1, A_2 = zero-time plasma drug concentration intercepts of regression lines of distribution phases I and II, respectively; B = zero-time plasma drug concentration intercepts of regression line of elimination phase; α_1,α_2 = rate constants of distribution phases I and II respectively; β = overall elimination rate constant; $t_{1/2\,a1}$, $t_{1/2\,a2}$ = half –lives of distribution phases I and II respectively; $t_{1/2\beta}$ = elimination half life; K_{12},K_{21} = rate of transfer of drug from central (blood) to peripheral (tissues) compartment I and vice-versa; K_{13}/K_{31} = rate of transfer of drug from central (blood) to peripheral compartment II, 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.

buffalo calves [5] has been reported. The high values of distribution rate constant α_1 (12.0 ± 1.55 /h) and α_2 (2.36 ± 0.15 /h) indicate that ceftriaxone was rapidly distributed into various body fluids and tissue compartments. The rapid distribution of ceftriaxone was further substantiated by high values of K_{13}/K_{31} (2.30 ± 0.48) and K_{12}/K_{21} (0.63 ± 0.14). The values of $V_{d(area)}$ of ceftriaxone in healthy animals [6] is higher (1.40 ± 0.07 l/kg) as compared to febrile animals (1.21 ± 0.15 l/kg). In accordance to our present findings, Saini [15] reported a decrease in $V_{d(area)}$ of amikacin in febrile cow calves as compared to healthy subjects. A marked decrease in the values of $V_{d(area)}$ during fever and other diseased conditions has also been reported for trimethoprim and chloramphenicol [4].

Table 3. Calculated intravenous dosage regimen of ceftriaxone required to maintain specified plasma ceftriaxone concentration in febrile buffalo calves

Desired plasma concentration (μg/ml)	Dosage interval (h)	Priming dose (mg/kg)	Maintenance dose (mg/kg)
0.2	6	1.90	1.64
0.2	8	3.82	3.58
0.4	6	3.80	3.28
0.4	8	7.64	7.16
0.6	6	5.70	4.92
0.6	8	11.5	10.7
0.8	6	7.60	6.56
0.8	8	15.3	14.3
1.0	6	9.50	8.20
1.0	8	19.1	17.9

In the present study, the calculated values of AUC in febrile buffalo calves were lower than the values reported in our earlier study [6] in healthy animals. Similarly, lower values of AUC for ceftriaxone in typhoid fever in man [1] and cefotaxime in buffalo calves [16] has been reported as compared to their respective healthy subjects. While comparing the total body clearance in febrile animals with that of healthy animals [6], it was found that the value of Cl_B in febrile animals (0.41 ± 0.03 l/kg/h) is significantly higher as compared to the healthy animals (0.26 ± 0.007 l/kg/h). Similarly, Acharya *et al*. [1] have also studied that Cl_B was increased in patients with typhoid fever as compared to their healthy subjects. Endotoxin causes hepatic, renal dysfunctions [24,25] as well as haemodynamic depression [21]. The depressing effect of endotoxin on the renal system could have been 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 drug during fever [18].

The ultimate objective 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 ceftriaxone to be used effectively in clinical practice for the treatment of mild to severe bacterial infections, without having first conducted a detailed pharmacokinetic study. Thus appropriate dosage schedule of ceftriaxone on the basis of pharmacokinetic data was calculated for buffalo in febrile conditions. With a minimum therapeutic plasma concentration of ceftriaxone as 1.0 μg/ml [13] 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 ceftriaxone in the febrile buffalo calves after intravenous administration would be 19 mg/kg followed by 18 mg/kg at 8h intervals.

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