The pharmacokinetics of cefepime in *E. coli* lipopolysaccharide induced febrile buffalo calves

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ABSTRACT

The pharmacokinetics of cefepime after its single intravenous administration (10 mg/kg) was investigated in experimentally induced fever in buffalo calves (n = 4). The fever was induced by single/ repeated intravenous injection of *E. coli* lipopolysaccaride (1 µg/kg). The drug was estimated in plasma samples by microbiological assay using *E. coli* (MTCC 739) test organism. The pharmacokinetic behaviour of cefepime in febrile animals was described by a two compartment open model. At 1 min, the concentration of cefepime in plasma was 40.8 ± 0.98 µg/mL which rapidly declined to 23.0 ± 0.64 µg/mL at 15 min. The drug was detected up to 24 h. The elimination half-life and volume of distribution were 3.00 ± 0.18 h and 0.42 ± 0.02 L/kg, respectively. The distribution half-life, AUC and total body clearance (Cl_B) were 0.08 ± 0.002 h, 101 ± 7.65 µg/mL.h and 98.8 ± 6.06 mL/kg/h, respectively. To maintain a minimum therapeutic concentration of 1 µg/mL, a satisfactory dosage regimen of cefepime in febrile buffalo calves would be 7 mg/kg repeated at 12 h intervals.

Key words: buffalo calf, cefepime, dosage regimen, fever, pharmacokinetics

Introduction

Cefepime, is a parenteral semisynthetic bactericidal cephalosporin having excellent activity against a broad spectrum of clinically important pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains resistant to other broad spectrum cephalosporins (BARBHAIYA et al., 1992). The pharmacokinetics of chemotherapeutic agents are markedly altered in disease conditions (LESAR and ZASKE 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 always extrapolated in clinical cases to treat diseased animals. Fever,

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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 (MONSHOUWER et al., 1996). So, a study of the influence of fever on the pharmacokinetics of antibiotics is essential. The purpose of this study was to determine the pharmacokinetic variables of cefepime in febrile buffalo calves following intravenous administration. From the pharmacokinetic data, recommendations were made for the optimal dosage regimen of cefepime in febrile buffalo calves.

Materials and methods

The experiment was performed on four male buffalo calves, 6-10 months of age and weighing an average of 91 kg. The animals were housed in a departmental shed with a concrete floor and were provided green fodder and water ad libitum. Each animal was quarantined for three weeks before the start of the experiment and was determined to be healthy by regular clinical examination. Fever was induced by single/repeated intravenous administration of *E. coli* lipopolysaccaride (LPS) at the dose rate of 1µg/kg body mass (DARDI et al., 2005). Once fever was induced (1-2 h after LPS injection) cefepime was injected intravenously to these four animals at a dose rate of 10 mg/kg of cefepime, in a 10% solution with sterilized distilled water. Blood samples (4-5 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 and 60 minutes and 2, 3, 4, 5, 6, 8, 10, 12, 16 and 24 h after administration of the drug. Plasma was collected after centrifugation at 2000g for 15 minutes at room temperature and kept at -20^0 C until analysis, usually the next day. The concentration of cefepime in plasma was estimated by employing the 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. However, it measured the overall microbiological activity of the drug. The standard curve of cefepime equivalent in buffalo calf plasma was linear between 0.25 to 1.25 µg/mL. The drug could be detected up to a minimum limit of 0.1 µg/mL. The intra day and inter day coefficient of variance was 4.2 and 9.39 per cent, respectively. The plasma concentration-time data for each buffalo calf were determined according to the computed least squares regression technique. The kinetic parameters were calculated from the formulae derived for a two- compartment open model (NOTARI, 1980; GIBALDI and PERRIER, 1982).

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

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

Where $C_p(min)$ is the minimum therapeutic concentration of cefepime, τ is the dosage interval and other parameters are defined in Table 1.

Results

The effect of *E. coli* lipopolysaccharide on body temperature was recorded. The normal temperature in the buffalo calves was in the range of 37.1-37.6 ºC. In one animal, there was a significant rise in body temperature within 2 h of administration of lipopolysaccharide, while in three animals, the dose of lipopolysaccharide was repeated to obtain an optimum increase in body temperature. This dose of lipopolysaccaride caused fever within two hours and fever persisted for 7-10 hours. At least a 1.1 ºC increase of temperature from the normal temperature was taken as the time of cefepime administration.
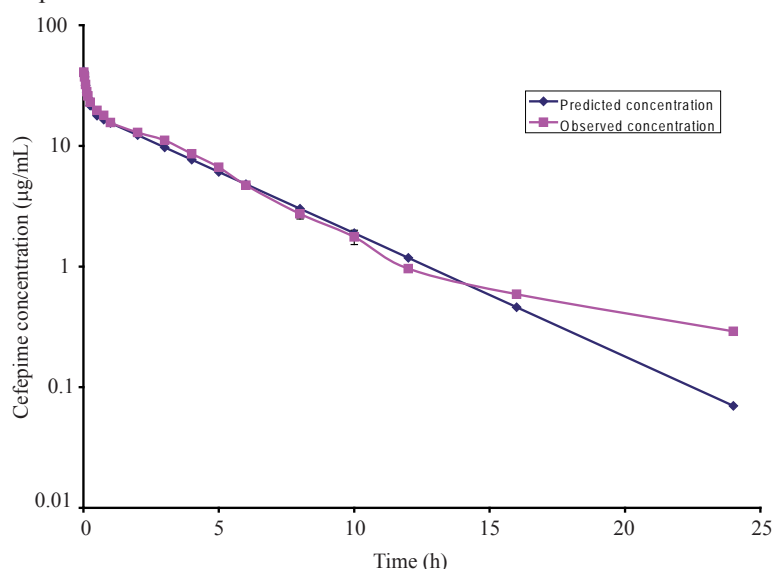

Fig. 1. A semi logarithmic plot of predicted and observed plasma levels of cefepime after a single intravenous dose of 10 mg/kg body mass in febrile buffalo calves. Values given are mean ± SE of four animals.

The mean plasma concentrations of cefepime as a function of time in the febrile buffalo calves were plotted on a semilogarithmic scale (Fig. 1). At 1 minute the mean plasma concentration of cefepime was 40.8 ± 0.98 µg/mL, which rapidly declined to plasma concentration of 23.0 ± 0.64 µg/mL at 15 minutes. Then levels gradually decreased to 0.29 ± 0.01 µg/mL at 24 hours. The elimination half life ($t_{1/2\beta}$), volume of distribution ($Vd_{(area)}$) and total body clearance in febrile animals were 3.00 ± 0.18 h, 0.42 ± 0.02 L/kg and 98.8 ± 6.06 mL/kg/h, respectively. Various pharmacokinetic parameters for cefepime in buffalo calves, in which fever was induced before administration of the drug, are given in Table 1.

Table1. Pharmacokinetic parameters of cefepime in febrile buffalo calves (n = 4) after a single intravenous injection of 10 mg/kg body mass

Parameter[a]	Unit	Mean ± SE
C_p^o	μg/mL	44.9 ± 1.49
A	μg/mL	25.3 ± 1.70
B	μg/mL	19.6 ± 0.84
α	h^{-1}	8.35 ± 0.29
β	h^{-1}	0.234 ± 0.015
$t_{1/2\alpha}$	h	0.08 ± 0.002
$t_{1/2\beta}$	h	3.00 ± 0.18
K_{12}	h^{-1}	4.36 ± 0.28
K_{21}	h^{-1}	3.77 ± 0.20
$K_{12/K21}$ ratio	-	1.17 ± 0.11
AUC	μg/mL.h	101.0 ± 7.65
$V_{d(area)}$	L/kg	0.42 ± 0.02
$V_{d(SS)}$	L/kg	0.48 ± 0.02
Cl_B	mL/kg/h	98.8 ± 6.06

[a] Kinetic parameters as described by Gibaldi and Perrier (1982). C_p^o = Intercept of the back extrapolated concentration time curve on the concentration axis; 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, based on area under curve; $V_{d(ss)}$ = apparent volume of distribution, based on steady state plasma levels; Cl_B = total plasma clearance.

Discussion

Evaluation of the results of plasma cefepime levels against time indicated that the pharmacokinetics of cefepime 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 cefepime 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 the natural logarithm. The disposition of cefepime, following intravenous injection, has been reported to follow the 2-compartment open model in calves (ISMAIL, 2005a; PAWAR and SHARMA, 2008) and ewes (ISMAIL, 2005b), horses (GUGLICK et al.,

1998; GARDNER and PAPICH, 2001) buffalo calves (JOSHI and SHARMA, 2007) and dogs (NAKANOMYO et al., 1992).

A comparison of plasma levels of cefepime in febrile animals with healthy animals (JOSHI and SHARMA, 2007), indicated that the peak plasma levels of cefepime in febrile buffalo calves (40.8 ± 0.98 μg/mL) was almost identical to healthy buffalo calves (42.7 ± 1.85 μg/mL). The high values of the distribution rate constant α (8.35 ± 0.29 h^{-1}) indicates that cefepime is rapidly distributed into various body fluids and tissue compartments. The rapid distribution of cefepime is further substantiated by high values of K_{12}/K_{21} (1.17 ± 0.11). The elimination half-life of cefepime in febrile animals (3.00 ± 0.18 h) is similar to that reported in healthy animals, but longer than the values reported in other species. The elimination half-life of cefepime had been reported as 2.67 ± 0.29 h in healthy buffalo calves (JOSHI and SHARMA, 2007), 2.38 h in calves (ISMAIL, 2005a), 1.76 h in ewes (ISMAIL, 2005b), 2.1 h in horses (GUGLICK et al., 1998), 2 h in humans (KIEFT et al., 1993), 1.65 h in foals and 1.09 h in dogs (GARDNER and PAPICH, 2001). The calculated value of volume of distribution ($V_{d(area)}$) in febrile buffalo calves (0.42 ± 0.02 L/kg) is more than the reported value of $V_{d(area)}$ (0.225 L/kg) in horses (GUGLICK et al., 1998). The value of $V_{d(area)}$ in buffalo calves suggested that cefepime is distributed principally through extracellular fluid space. Despite low $V_{d(area)}$ values for cefepime in most species, its efficacy against infections located in barrier restricted compartments, such as the CNS, has been documented (GRASSI and GRASSI, 1993). Apparently, the low degree of plasma protein binding and the probable decrease in integrity of the blood brain barrier caused by inflammation promote the attainment of therapeutic concentrations of cefepime in the brain tissue (GUGLICK et al., 1998). The value of $V_{d(ss)}$ in buffalo calves (0.48 ± 0.02 L/kg) also indicated good extravascular distribution of the drug. As with other cephalosporins, the extravascular distribution of cefepime was limited to the extracellular fluid (BALANT et al., 1985). In spite of its limited distribution into the extracellular fluid, as reported in most species studied, it appears that low protein binding and high bacterial activity are considered the major determinants for promotion of its therapeutic efficacy in all species (KALMAN et al., 1992).

While comparing the total body clearance in febrile animals with that of healthy animals (JOSHI and SHARMA, 2007), it was found that the value of Cl_B in febrile animals (98.8 ± 6.06 mL/kg/h) is almost identical to that of healthy animals (86.1 ± 3.65 mL/kg/h). Endotoxin causes hepatic and renal dysfunction (WILKINSON, 1977) as well as hemodynamic depression (VAN MIERT, 1973). Due to 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). Cefepime is not significantly metabolized, but is excreted unchanged, primarily in urine by the glomerular filtration process, and is poorly bound to plasma proteins (BARBHAIYA et al.,

1992), and these may be the reasons why febrile conditions did not affect the pharmacokinetics of cefepime.

The ultimate objective of the present study was to determine a satisfactory dosage regimen in febrile buffalo calves. It is not axiomatic to be able to compute the dosage regimen of cefepime 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 cefepime 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 cefepime in the febrile buffalo calves after intravenous administration would be 7 mg/kg, repeated at 12h intervals. The dosage schedule of cefepime reported in healthy buffalo calves (JOSHI and SHARMA, 2007) and febrile calves (PAWAR and SHARMA, 2008) was 8 mg/kg, repeated at 12h and 8.2 mg/kg repeated at 8h, respectively. Antibiotic dosing regimens have traditionally been determined by pharmacokinetic parameters only. However, pharmacodynamics play an equal role, because pharmacodynamic parameters may be used to design dosing regimens, which counteract or prevent resistance. Integrating the pharmacokinetic parameters with the MIC gives pharmacokinetic/pharmacodynamic (PK/PD) parameters (T>MIC, Cmax/MIC, AUC/MIC), which quantify the activity of an antibiotic. The pattern of activity for cephalosporins is time dependent killing and minimal persistent effects. The ideal dosing regimen for these antibiotics maximizes the duration of exposure. The T>MIC is the parameter that best correlates with efficacy. For these drugs, maximum killing is seen when the time above MIC is at least 70 per cent of the dosing internal (HYATT et al., 1995). The T>MIC values (10 h) of cefepime suggested that this agent is clinically effective in the treatment of various infections manifested with fever.

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SAŽETAK

Istražena je farmakokinetika cefepima u bivolske teladi (n = 4) s pokusno izazvanom vrućicom nakon njegove jednokratne intravenske primjene (10 mg/kg). Vrućica je bila izazvana jednokratnom ili ponovljenom intravenskom primjenom lipopolisaharida bakterije *E. coli* (1 μg/kg). Farmakokinetika lijeka određivana je u uzorcima plazme mikrobiološkim postupkom upotrebom soja *E. coli* (MTCC 739). Farmakokinetičko ponašanje cefepima u febrilnih životinja opisano je na osnovi dva otvorena modela. U prvoj minuti koncentracija cefepima u plazmi iznosila je 40,8 ± 0,98 μg/mL, a u 15. se naglo smanjila na 23,0 ± 0,64 μg/mL. Lijek je bio dokazan do 24 sata nakon davanja. Poluvrijeme njegova izlučivanja iznosilo je 3,00 ± 0,18 h, a količina raspodjele 0,42 ± 0,02 L/kg. Poluvrijeme njegove raspodjele iznosilo je 0,08 ± 0,002 h, površine ispod koncentracijske krivulje u plazmi 101 ± 7.65 μg/mL, a ukupni klirens iz organizma (Cl_B) 98,8 ± 6,06 mL/kg/h. Za održavanje minimalne terapijske koncentracije od 1 μg/mL cefepim bi u febrilne bivolske teladi trebalo davati u dozi od 7 mg/kg s ponovljenom primjenom u razmaku od 12 sati.

Ključne riječi: bivolska telad, cefepim, doziranje, vrućica, farmakokinetika