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RESEARCH ARTICLE

Pharmacokinetics of Cefepime Following Intravenous and Intramuscular Administration in Sheep

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ABSTRACT

Pharmacokinetics of cefepime was studied following single dose intravenous and intramuscular administration at the dose of 20 mg/kg of body weight in sheep. Drug concentration in serum was determined using high performance liquid chromatography (HPLC). Following single dose intravenous administration, the drug was rapidly distributed ($t_{1/2\alpha}$: 0.20 ± 0.02 h) and eliminated ($t_{1/2\beta}$: 2.54 ± 0.12 h) from the body. The area under curve ($AUC_{0-\infty}$) was 135.50 ± 5.63 μg h/mL. The drug was cleared at the rate of 2.48 ± 0.09 mL/min/kg with mean residence time (MRT) of 2.84 ± 0.13 h. Following IM administration, the drug was rapidly absorbed (C_{max}: 26.34 ± 1.44 μg/mL; t_{max}: 0.75 h) and slowly eliminated ($t_{1/2\beta}$: 5.17 ± 0.44 h) from body. The volume of distribution at steady state (Vd_{ss}), area under curve (AUC), total body clearance (Cl_B) and mean residence time (MRT) were 1.11 ± 0.10 L/kg, 140.90 ± 8.67 μg h/mL, 0.15 ± 0.01 mL/min/kg and 6.89 ± 1.0 h, respectively. The bioavailability of cefepime following intramuscular administration was 103 ± 8.0 %.

Keywords: *Pharmacokinetics, Cefepime, Sheep, Intravenous, Intramuscular*

Cefepime is an extended spectrum, semi-synthetic, parenteral fourth-generation cephalosporin antibiotic. It has excellent activity against gram-positive and gram-negative bacteria, except for *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus, Clostridium difficile, Bactereoides spp.* and some strains of *Pseudomonas spp.* [1, 2]. It has variable activity against anaerobic bacteria [3]. Cefepime was found highly active against canine isolates of *Staphylococcus intermedius, Pseudomonas aeruginosa* and *Echerichia coli* with MIC values of 0.03, 0.5, 1.0 μg/mL, respectively [4]. Pharmacokinetic profile of cefepime has been studied in monkey [5], cow calves [6-8], horses [9], foals and dogs [10], ewes [6] and goats [11-13]. Cefepime may be useful in cases of sub-clinical and clinical mastitis in sheep as it has been reported to be excreted in milk following intravenous and intramuscular administration in goats [12]. Findings from pharmacokinetic studies in animals encourage the use of cefepime to treat many types of bacterial infections in sheep. Therefore, the present study was planned to determine the pharmacokinetic parameters of cefepime following single dose intravenous and intramuscular administration in Patanwadi breed of sheep.

MATERIALS AND METHODS

Experimental Animals

The experiment was conducted on six healthy Patanwadi female sheep (4-5 years old), weighing 25-30 kg. The study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai (India). The animals were examined clinically to evaluate health status. Each animal was housed in a separate pen and provided standard ration. Water was provided *ad libitum.*

Drugs and Chemicals

Pure cefepime powder was obtained from Aurobindo Pharma Ltd., Hyderabad, India. Cefepime hydrochloride (Novapime, Lupin Ltd., Mumbai, India) equivalent to 1 g cefepime was purchased from pharmacy. Water, sodium acetate, acetic acid, acetonitrile and trichloroacetic acid of analytical or

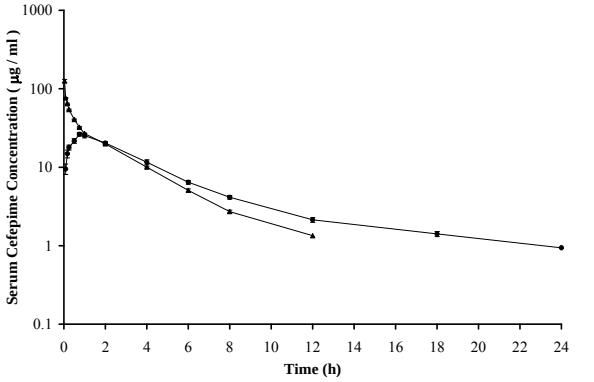

Fig 1. Semilogarithmic plot of cefepime concentrations in serum versus time following single dose intravenous and intramuscular administrations at the rate of 20 mg/kg of body weight in sheep. Each point represents the mean ± S.E. of six animals.

HPLC-grade were procured from Merck India Ltd., Mumbai.

Experimental Design

Cefepime hydrochloride was dissolved in sterile water to make a final concentration of 100 mg/mL and 200 mg/mL for intravenous and intramuscular administrations, respectively at a dose of 20 mg/kg body weight. The animals were randomized and experiment was planned in crossover design. An interval of 21 days was observed between two successive injections. Intravenous injection of the drug was given through left jugular vein and intramuscular injection was given in deep gluteal muscle using a 20 G × 25 mm needle. Blood samples (2-3 mL) were collected through the intravenous catheter fixed in right jugular vein into heparinized glass test tubes before administration and at 2, 5, 10, 15, 30, 45 min and 1, 2, 4, 6, 8, 12, 18, and 24 h after intravenous administration while blood samples were collected before administration and at 5, 10, 15, 30, 45 min and 1, 2, 4, 6, 8, 12, 18, 24 and 36 h after intramuscular administration of the drug. The animals were observed for any side effects during the study after administration of the drug. The blood samples were allowed to clot and serum was harvested from all samples. The serum samples were stored at – 40°C and assayed within a week.

Cefepime Assay

Cefepime concentration in serum samples was determined by reverse-phase high performance liquid chromatography (HPLC) assay [10] with minor modification. The HPLC system (Laballiance, USA) comprised of gradient solvent delivery pump (model AIS 2000) and UV detector (model 500). Chromatographic separations were performed by using reverse phase C18 column (Thermo, 5 µ ODS; 250 × 4.6 mm ID) at room temperature. Data integration was performed using Clarity (Version 2.4.0.190). The mobile phase was a mixture of 0.2 M sodium acetate (3.2%), 0.2 M acetic acid (2.2 %), acetronitrile (15.0 %) and HPLC water (79.6 %) with pH 5.1. Mobile phase was filtered through a 0.45 µ filter and pumped into column at a flow rate of 1.0 mL/min at ambient temperature. The elute was monitored at 257 nm wavelength. Serum samples were deproteinized by diluting 500 µl of serum with 500 µl of trichloroacetic acid (10%) and centrifuged at 5000 revolution per minute for 10 minutes. The clean supernant was collected and an aliquot of 20 µl of the supernant was injected into the loop of HPLC system through manual injector.

Calibration curve was prepared by adding known amount of cefepime to blank unfortified serum for the expected range of concentrations from 0.1 to 200 µg/mL and processed as described above. Quantification was done by reference to the resultant calibration curves. The calibration curve was prepared daily and not accepted unless it had a R^2 value ≥ 0.99. Recovery from serum was estimated by comparing the detector response of series of standards (0.1 to 200 µg/mL) extracted from serum with non extracted standards in mobile phase. Recovery of the drug from serum samples was estimated to be 88.3 %. The lower limit of quantitation was 0.1 µg/mL. The assay was sensitive, reproducible and linearity was observed from 0.1 to 200 µg/mL. The retention time of cefepime was 4.0 minutes.

Pharmacokinetic Analysis

Following intravenous administration of the drug, the drug concentration-time data were best fitted to two compartment open model, where as drug concentration-time data following intramuscular administration were analyzed by non-compartment technique. Following formulas were used to calculate various pharmacokinetic parameters [14-16].

a) Half-life

i) $t_{½\ \alpha} = 0.693 / \alpha$

ii) $t_{½\ \beta} = 0.693 / \beta$

b) AUC (0 - ∞), 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 (ss), the volume of distribution of drug at steady state:

$Vd(ss) = Dose \times AUMCm / (AUC)^2$

d) Cl_B, the total body clearance of drug:

$Cl_B = \beta \times Vd(ss) \times 1000$

e) MRT, the mean residence time:

MRT = AUMC / AUC

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

$F = (t_{½\ \beta}\ (I.V.) \times AUC\ (I.M.)) / (t_{½\ \beta}\ (I.M.) \times AUC\ (I.V.))$

RESULTS

Semilogarithmic plot of cefepime concentrations in serum versus time following single dose intravenous and intramuscular administrations (20 mg/kg) in sheep is shown in Fig 1. Cefepime pharmacokinetic parameters following intravenous and intramuscular administration in sheep are shown in Table 1. Following

Table 1. Pharmacokinetic parameters of cefepime after single dose intravenous and intramuscular administrations (20 mg/kg of body weight) in sheep

Pharmacokinetic Parameters*	Unit	Intravenous dose (Mean ± S.E., n = 6)	Intramuscular dose (Mean ± S.E., n = 6)
$t_{1/2\alpha}$	h	0.20 ± 0.02	-
$t_{1/2\beta}$	h	2.54 ± 0.12	5.17 ± 0.44
$AUC_{(0-\infty)}$	μg.h/mL	135.50 ± 5.63	140.90 ± 8.67
AUMC	$\mu g.h^2/mL$	386.70 ± 28.8	921.90 ± 116.0
Vd_{ss}	L/kg	0.42 ± 0.02	1.11 ± 0.1
$Cl_{(B)}$	mL/min/kg	2.48 ± 0.09	0.15 ± 0.01
MRT	h	2.84 ± 0.13	6.89 ± 1.0
F	%	-	103.0 ± 8.0
C_{max}	μg/mL	-	26.34 ± 1.44
T_{max}	h	-	0.75

*Pharmacokinetic Parameters: $t_{1/2\alpha}$: half-life of distribution phases; $t_{1/2\beta}$: elimination half life; $AUC_{(0-\infty)}$: total area under plasma drug concentration-time curve; AUMC: area under first of moment curve; Vd_{ss}: volume of distribution at steady state; Cl_B: total body clearance; MRT: mean residence time; F: bioavailability; C_{max}: maximum drug concentration; T_{max}: time of maximum concentration observed in serum

intravenous administration, the drug could not be detected in serum samples collected beyond 12 h. The distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) half lives of cefepime were 0.20 ± 0.02 h and 2.54 ± 0.12 h, respectively. The values of volume of distribution at steady state (Vd_{ss}), area under curve (AUC), and total body clearance (Cl_B) were 0.42 ± 0.02 L/kg, 135.50 ± 5.63 μg h/L, and 2.48 ± 0.09 mL/min/kg respectively.

Following single dose intramuscular administration of cefepime in sheep, the peak serum cefepime concentration of 26.34 ± 1.44 μg/mL was observed at 0.75 h. The elimination ($t_{1/2\beta}$) half life of cefepime was 5.17 ± 0.44 h. The average respective values for volume of distribution by area method (Vd_{ss}), area under curve (AUC) and total body clearance (Cl_B) were 1.11 ± 0.1 L/kg, 140.90 ± 8.67 μg h/L, and 0.15 ± 0.01 mL/min/kg. The systemic bioavailability (F) of the drug was calculated to be 103.0 ± 8.0 per cent in sheep following intramuscular administration.

DISCUSSION

Following intravenous administration, the drug gets rapidly eliminated as evidenced by short half-life and faster clearance. Similar observations have been reported in goats (10) and calves [17]. More rapid elimination of cefepime has been found in foals ($t_{1/2\beta}$: 1.65 ± 0.0.01 h) and dogs ($t_{1/2\beta}$: 1.09 ± 0.27 h) [10]. The drug has moderate distribution in the body of sheep. This is in agreement with the Vd_{ss} of 0.35 ± 0.03, 0.32 ± 0.01 and 0.43 ± 0.03 L/kg reported in goats [11] ewes [6] and calves [7], respectively.

Following single dose intramuscular administration of cefepime, the peak and minimum serum cefepime concentration observed in sheep is in agreement with the peak serum cefepime concentrations of 21.1 ± 1.85 and 22.6 μg/mL reported in goats [11,13]. However, lower peak serum concentration were observed in horses, cow calves and dogs [4, 8, 9], while higher peak plasma concentration of cefepime (31.9 ± 1.5 μg/mL at 1.1 ± 0.2 h) has been reported in ewes [6]. The elimination half-life ($t_{1/2\beta}$) following intramuscular administration of cefepime in sheep is in line with that of 4.89 ± 0.24 h reported in goats [11] but longer than that of 3.02 ± 0.18 h reported in calves [3]. Longer half-life indicates that the drug is continuously absorbed during the elimination phase in sheep following intramuscular administration. The total body clearance of cefepime in sheep is slower than observed in goats [11] and cow calves [8]. Following intramuscular injection of cefepime in sheep, the drug gets extensively distributed. However, moderate distribution of the drug has been reported in goats (0.60 ± 0.06) [11]. The drug was completely absorbed from site of injection following intramuscular administration in sheep as evidence by cent percent bioavailability. Similarly higher systemic bioavailability of 111.0 ± 22.0 and 98.0 ± 3.0 was reported in horses [9] and cow calves [8]. However, lower systemic bioavailability of 69.0 ± 6.0 was reported in goats [11].

The pharmacokinetic profile of cefepime in sheep following intravenous and intramuscular administration indicates that it may be therapeutically useful against susceptible micro-organisms involved in most common infections in sheep. The high bioavailability of cefepime and maintenance of therapeutic concentration for a long time following intramuscular injection suggests that cefepime is suitable for intravenous and intramuscular administration (20 mg/kg repeated at 18 h interval) for the treatment for systemic bacterial infections in sheep.

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