Pharmacokinetics of Cefpirome Following Intravenous and Intramuscular Administration in Goats

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ABSTRACT

The pharmacokinetic profile of cefpirome following intravenous and intramuscular administration (10 mg/kg) was evaluated in goats. Cefpirome concentration in plasma samples was determined by reverse-phase High Performance Liquid Chromatography. Following intravenous administration of the cefpirome in goats, volume of distribution at steady-state (Vd_{ss}), elimination half-life ($t_{1/2\beta}$) and total body clearance (Cl_B) were 0.35 ± 0.01 L/kg, 2.12 ± 0.14 h and 2.13 ± 0.05 ml/min/kg respectively. Following intramuscular administration of the drug, peak plasma concentration (C_{max}), elimination half-life ($t_{1/2\beta}$) and bioavailability (F) were 10.97 ± 0.34 µg/mL, 2.09 ± 0.08 h and 75 ± 4 %, respectively. Plasma protein binding of cefpirome in goat was found to be 19.9 %. Pharmacokinetic – pharmacodynamic integration indicates cefpirome can be useful in goats at dose rate of 10 mg/kg and repeated at interval of 12 hours by the intramuscular route.

Key Words: cefpirome, pharmacokinetics, intravenous, intramuscular, goat

INTRODUCTION

Cefpirome is a broad-spectrum semi-synthetic β-lactamase resistant fourth generation cephalosporin used in severely ill patients in the intensive care, oncology, and transplantation units (1, 2). It has increased affinity for penicillin binding proteins (3), reduces susceptibility to extended-spectrum β-lactamase (4) and has the ability to interfere with penicillin binding proteins mediated cell wall synthesis, ultimately leads to cell lysis (5). It has potent bactericidal activity against a broad range of gram-negative and gram-positive organisms, including *Pseudomonas aeruginosa* and methicillin susceptible *Staphylococcus* spp., *Haemophillus influenzae* type B and many members of the Enterobacteriaceae family (6).

Diseases like coliform septicaemia, pneumonia, colibacillosis and meningitis are major causes of neonatal mortality in goats where cefpirome can be used for treatment. The disposition kinetics of cefpirome have been investigated in rabbits (7), dogs (8, 9), mice, rats, and monkeys (9) and buffalo calves (10, 11). However, there is no information available on the pharmacokinetic of cefpirome in goats following intravenous and intramuscular administration. In order to understand species difference, the study was undertaken on pharmacokinetics of cefpirome following intravenous and intramuscular administration in the goat.

MATERIALS AND METHODS

Experimental Animals

The experiment was conducted on six healthy adult (2-3 years of age) Surti goats, weighing 28-32 kg. Each animal

was housed in a separate pen and provided a standard ration with *ad libitum* water. Goats were kept under constant observation for two weeks before the commencement of the experiment and subjected to clinical examination to exclude possibility of any diseases. The experimental protocol was approved by Institutional Animal Ethics Committee.

Drug and Chemical

Cefpirome technical grade powder was procured from Orchid Pharma Ltd., Chennai, India. Cefpirome sulpahte powder (1 g Ceforth®; Biochem pharmaceutical Industries Ltd., Mumbai, India) was purchased from a local pharmacy. Water, acetonitrile, acetic acid (HPLC grade) were purchased from Merck Millipore India Ltd., Marathe Marg, Mumbai, India. Sodium acetate was procured from S.D. Fine Chem. Ltd., Baroda, Gujarat, India, and perchloric acid (AR grade) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Drug Administration and Sample collection

All six animals were randomly allocated to receive either an intravenous or intramuscular injection of cefpirome at the dose rate of 10 mg/kg. A washout period of two weeks was observed between treatments. An intravenous injection of cefpirome was administered into the left jugular vein. Blood samples (3 mL) were collected through an intravenous catheter (Venflon, 22 × 0.9 × 25 mm, Becton, Dickinson and Company) fixed in the right jugular vein in heparinised glass test tubes, prior to injection and at 2, 5, 10, 15, 30 min and 1, 2, 4, 8 and 12 h after intravenous administration. Following intramuscular injection of cefpirome in the left deep gluteal muscle, blood samples (3 mL) were collected in heparinised glass test tubes after drug administration at 5, 10, 15, 30 min and 1, 2, 4, 8, 12, 18 and 24 h. Goats were monitored for any adverse reactions during the entire study period. Blood samples were centrifuged at 4116 g for 10 minutes at 4°C and plasma transferred to cryo-vials (2 mL) and, stored at – 20°C. Samples were analyzed within 48 h to quantify cefpirome concentration using High Performance Liquid Chromatography.

Analytical assay of cefpirome and pharmacokinetic analysis

Cefpirome concentration in plasma samples was determined by reverse-phase High Performance Liquid Chromatography (HPLC) after extraction, using a reported assay (12) with minor modifications. The High Performance Liquid Chromatography (HPLC) apparatus of Laballiance (USA) which was comprised of quaternary gradient delivery pump (model AIS 2000), UV detector (model 500) and C18 column (PARTISIL 5 ODS-3 RAC II, 4.6 × 100 mm ID) was used. Pharmacokinetic data integration was carried by software "Clarity" (Version 2.4.0.190). Plasma (500 µL) was deproteinized by addition of perchloric acid (0.8 M) and methanol (50:50) and vortexed for one minute. This was followed by centrifugation at 4116 g for 10 minutes. An aliquot of supernatant was collected in a clean vial and 20 µL was injected into the loop of HPLC system. The mobile phase was a mixture of 0.2 M sodium acetate (4%), acetronitrile (20.0 %) and HPLC water (76 %). 0.2M acetic acid was used to adjust pH of 5.1. The mobile phase was filtered by 0.45 µ filter and pumped into column at a flow rate of 1.0 mL/min at ambient temperature. The effluent was monitored at 258 nm wavelength.

A calibration curve was prepared daily for drug concentration ranging from 0.16 to 20 µg/mL. The assay was sensitive (Lower Limit of Detection: 0.16 µg/mL) and reproducible, and linearity was observed from 0.16 to 20 µg/mL (r^2 = 0.99). Precision and accuracy were determined using quality control (QC) samples at concentrations 1, 5, 20 µg/mL (5 replicates each day). Intraday and interday coefficients of variability for five QC samples were satisfactory with the relative deviations (RSD) of less than 5.04 %. The absolute recovery of cefpirome was measured up to 89.12 %. Various pharmacokinetic parameters were calculated from plasma concentration of cefepime using software PK solution (version 2.0). The bioavailability (F) was calculated using following formula: F % = AUC (IM) / AUC (IV).

Plasma Protein Binding

Blood was collected from jugular vein into heparinised test tubes and was centrifuged at 4116 g for 5 min. The plasma was transferred into clean vials and stored at -20 ^{0} C and analysed within 24 hour. Various concentrations of cefpirome, 1.25, 5 and 20 µg/ml were prepared in goat plasma in triplicates. The plasma samples were mixed and incubated for 1 hour at 37°C. Thereafter, plasma (200 uL) was transferred to each of the Microcon-10 Ultrafiltration assemblies, labeled properly and centrifuged at 2000 g at 37°C for 45 minutes. The assembly was removed from the centrifuge, the filter was

removed along with protein rich plasma, and transferred to a new centrifuge vial kept in an upright position to recover protein rich plasma. Samples were re-centrifuged at 2000 X g at 37 ^{0} C for 2 minutes (13). The resultant supernatant and the filtrate samples were analyzed by reverse phase high performance liquid chromatography as per the procedure mentioned above. The percentage unbound drug was calculated as per following equation: % Plasma protein binding (PBB)

$$PPB\ \% = 100 - \frac{\text{Concentration in ultrafiltrate}}{\text{Concentration added}} \times 100$$

Statistical Analysis

Cefpirome serum concentration and pharmacokinetic parameters of different treatment groups were compared by students' "t" test using SPSS software (version 12.0.1). Statistical differences were considered at $p \leq 0.05$.

RESULTS

Following single dose intravenous and intramuscular administration of cefpirome in the goats, adverse reactions were not observed. The mean plasma concentration-time profile of cefpirome following intravenous and intramuscular administration at 10 mg/kg body weight has been presented graphically in Figure 1. Pharmacokinetic parameters (Mean ± SE) calculated after both route of drug administration have been depicted in Table 1.

Following intravenous administration of cefpirome, the plasma drug concentration rapidly declined from 64.30 ± 0.95 µg/mL (2 min) to 16.54 ± 0.46 µg/mL (1 h) (Figure 1). Thereafter, the drug concentration (1.67 ± 0.14 µg/mL) in plasma diminished gradually and was detectable for up to 8 h. The drug was not detected in plasma samples collected beyond 8 h post intravenous administration in goats. Therapeutic concentration of cefpirome ≥ 0.5 µg/mL was maintained in plasma from 5 minute to 8 h after intravenous administration. Following intramuscular administration of the cefpirome, mean peak plasma drug concentration (C_{max}) of 10.84 ± 0.39 µg/mL was achieved at 0.5 h (T_{max}) which declined rapidly to 7.55 ± 0.32 µg/mL at 2 h. The drug concentration of 0.75 ± 0.05 µg/mL in plasma was detected at 12 h and thereafter drug was not detected in plasma samples collected beyond 12 h post intramuscular administration in goats.

Following intravenous administration of the drug in goats, distribution half-life ($t_{1/2\alpha}$) ranged from 0.81 and 1.95 h with a mean of 1.51 ± 0.19 h. The mean values of apparent volume of distribution at steady-state (Vdss) were calculated to be 0.35 ± 0.01 L/kg. The range of elimination rate constant (β) was 0.26 to 0.39 h^{-1} with a mean of 0.33 ± 0.02 h^{-1}. The elimination half-life ($t_{1/2\beta}$) ranged from 1.77 to 2.63 h

Table1: Pharmacokinetic parameters of cefpirome (10 mg/kg) following intravenous and intramuscular administration in goats. (Mean ± SE, n=6).

Pharmacokinetic parameter	Unit	Intravenous Mean ± S.E	Intramuscular Mean ± S.E
K_a	h^{-1}	-	0.57 ± 0.02
β	h^{-1}	0.33 ± 0.02	0.33 ± 0.01
$t_{½Ka}$	h	-	1.22 ± 0.05
$t_{½\alpha}$	h	1.51 ± 0.19	-
$t_{½\beta}$	h	2.12 ± 0.14	2.09 ± 0.08
C_{max}	µg/mL	-	10.97 ± 0.34
T_{max}	h	-	0.54 ± 0.04
$AUC_{(0-\infty)}$	µg.h/mL	78.48 ± 1.94	58.30 ± 1.93
Vd_{ss}	L/kg	0.35 ± 0.01	-
$Cl_{(B)}$	ml/min/kg	2.13 ± 0.05	-
MRT	h	2.72 ± 0.11	4.27 ± 0.05
F	%	-	75.00 ± 4.00

K_a: absorption rate constant; β: elimination rate constant; $t_{1/2\alpha}$: half-life of distribution phases; $t_{1/2\beta}$: elimination half life; $t_{1/2k(a)}$: absorption half-life; $AUC_{(0-\infty)}$: total area under plasma drug concentration-time curve; V_{dss}: volume of distribution at steady state; Cl_B: total body clearance; MRT: mean residence time; C_{max}: maximum drug concentration; T_{max}: time of maximum concentration observed in plasma; F: bioavailability.

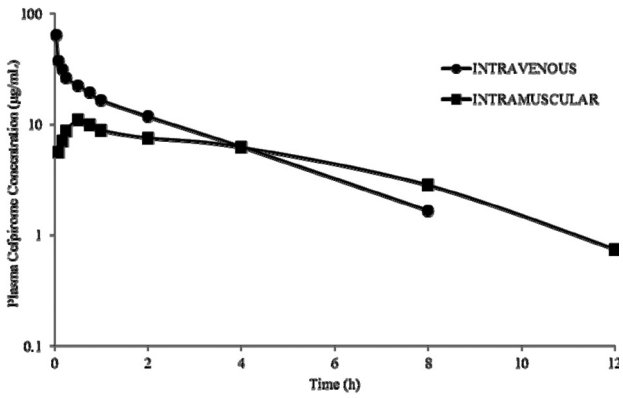

Figure 1: Semi-logarithmic plot of cefpirome plasma concentration after intravascular and intramuscular administration (10mg/kg) in goats. Each point represents mean of six animals.

with a mean of 2.12 ± 0.14 h. Total body clearance (Cl_B) of the drug was 2.13 ± 0.05 ml/min/kg with mean residence time (MRT) of 2.72 ± 0.11 h. Following intramuscular administration of the drug, absorption ($t_{½Ka}$), elimination half-life ($t½\beta$) were 1.22 ± 0.05 and 2.09 ± 0.08 h, respectively. The bioavailability (F) of the drug following intramuscular administration ranged from 59 to 85 % with an average of 75 ± 4 %.

DISCUSSION

Elimination half life ($t_{1/2}$: 2.12 ± 0.14 h) of cefpirome following single dose intravenous administration observed in goats is in agreement to the elimination half life of 2.14 ± 0.02 h reported in buffalo calves (10). However shorter elimination half life of 1.05 h and 0.90 h in dogs (8,9) and 1.17 h in monkeys and 1.48 h in rabbits (9) have been reported. The mean residence time calculated following single dose intravenous administration was 2.72 ± 0.11 h in goats, which is in agreement with reported values of 2.89 ± 0.01 h in buffalo calves (10). However it was observed that cefpirome principally eliminated by the kidney and 80-90% of the administered drug was recovered as unchanged form in the urine (7,14). Faster body clearance (2.13 ± 0.05 ml/min/kg) of cefpirome in goats following intravenous route of administration was obtained in the present study. Similarly higher clearance values of 2.00 ± 0.05 ml/min/kg in buffalo calves (10) and 3.2 ml/min/kg in dogs (8) have been reported following intravenous administration of cefpirome. Moreover cefpirome bound to plasma proteins of goats to the extent of 19.9 % and this value in agreement to value reported in mouse (15%) and rat (13%) (9). Whereas, lower plasma protein binding values of 4, 7 and 8 % has also been reported in rabbit, dog and monkey, respectively (9). The low mean volume of distribution at steady state (Vd_{ss}: 0.35 ± 0.01 L/kg) in goats following cefpirome intravenous administration indicated the limited distribution of drug into various body fluids and tissues. Similar low value of Vd_{ss} (0.40 ± 0.004 L/kg) have been reported in buffalo calves (10). In addition to this distribution of other cephalosporins like cefepime was also reported in goats in a similar pattern (15,16).

Following intramuscular administration of cefpirome in goats, the peak plasma concentration (C_{max}) of 10.97 ± 0.34 µg/ml was found at 0.5 h (T_{max}). Similarly C_{max} of 9.04 ± 0.5 µg/ml at 0.5 h in buffalo calves (10), 11.7 µg/ml at 0.42 h in monkeys and 15.4 µg/ml at 0.70 h in dogs (9) have been reported. Elimination half-life ($t_{½\beta}$: 2.09 ± 0.08 h) obtained in present study is in agreement elimination half-life of 2.39 ± 0.05 h reported in buffalo calves (11). However shorter elimination half life of 1.38 h in dogs and 1.23 h in monkeys (9) were also reported. Systemic bioavailability (75 ± 4 %) following intramuscular administration of cefpirome in goats was found higher than 35.3 ± 3.1 % as reported in buffalo calves (11). High systemic bioavailability and maintenance of therapeutic concentration up to 12 h following intramuscular injection suggests that cefpirome is suitable for intramuscular administration for the treatment for systemic bacterial infections in goats.

For β-lactam antibiotics, the time for which serum drug concentration exceeds the Minimum Inhibitory Concentration (MIC) (T>MIC) of pathogens is considered as primary determinant of antibacterial efficacy (17). In addition to this, for β-lactam antibiotics maximum killing was seen when the time above MIC is at least 70 percent of the dosing interval (18). Minimum inhibitory concentration for a majority of cefpirome sensitive bacteria like *Staphylococcus aureus* and *Escherichia coli* in the range of 0.1 to 0.5 µg/mL (19, 20). When integrating cefpirome pharmacokinetic data and the MIC values, cefpirome (10 mg/kg) needs to be administered intramuscularly at intervals of 12 hours.

In conclusions, integration of pharmacokinetics data generated from the present study with MIC range (0.1 to 0.5 µg/mL), cefpirome can be administer following intramuscular route at dose of (10 mg/kg) repeated at 12 h interval to maintain plasma concentration above MIC range.

CONFLICT OF INTEREST

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