INTRODUCTION
Cefoperazone, is a semi-synthetic third generation, piperazin β-lactam antibiotic that possesses broad spectrum activity against a wide range of aerobic and anaerobic gram-positive and gram-negative bacteria. Cefoperazone is highly effective against gram-positive, gram-negative and anaerobic bacteria including H. influenza, Neisseria meningitides and Streptococcus pneumonia. Cefoperazone is suitable for the treatment of bone and joint infections of horses (1), calf diseases such as diarrhea and pneumonia associated with gram-negative bacteria resistant to many commonly used antibiotics (2), intensive care infections of human beings (3) and has good penetration into the pancreas indicating its usefulness for the prophylaxis and therapy of secondary pancreatic infections (4). The pharmacokinetics of Cefoperazone has been determined in dog (5), calves (6), unweaned (7) and weaned (8) calves, buffalo calves (9, 10), horses (11) and human beings (12). Owing to its high efficacy, broad spectrum of activity, rapid tissue penetration, high safety and very low development of bacterial resistance, cefoperazone is gaining popularity among practitioners. Currently, it is extensively used in human medicine but increasing number of pharmacokinetic studies are now being undertaken in animals with a view to adopt the drug in veterinary medicine as well. Therefore, the present study was planned to determine the pharmacokinetics of cefoperazone in sheep following single respective intravenous and intramuscular administrations at the dose of 20 mg/kg body weight.
MATERIALS AND METHODS

Experimental Animals
The experiment was conducted on six Patanwadi sheep of 2-3 years age, weighing between 21 and 30 kilograms. The work was carried at Instructional farm, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, India. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and constituted by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. Animals were kept under constant observation for two weeks prior to commencement of the experiment and examined clinically to establish health status and to rule out the possibility of any diseases. Each animal was housed in a separate pen and provided standard ration. Water was provided ad libitum.

Drugs and Chemicals
Cefoperazone sodium (Megnamycin, Pfizer International Ltd., Mumbai, India) equivalent to Cefoperazone 1 gram for injection was procured from the local market. Potassium dihydrogen phosphate (KH$_2$PO$_4$, AR grade), perchloric acid (70 % about 1.67, GR), acetonitrile, methanol and water (HPLC grade) were purchased from Hi Media Laboratories Pvt.Ltd. and Merck India Ltd., Mumbai, India.

Experimental Design
The study was conducted in a cross over design with an interval of fifteen day between two successive injections. All sheep were randomly allocated to receive either intravenous (IV) or intramuscular (IM) injection. Cefoperazone sodium 1 g (Megnamycin, Pfizer International Ltd., Mumbai) was diluted with sterile water to make total volume of 10 ml and administered at a dose rate of 20 mg/kg body weight. IV injection of the drug was given in the left jugular vein, while IM injection was given in the deep gluteal muscles, using a 20 G × 25 mm sterile needles.

Collection of samples
Blood samples (2 mL each) were collected using an intravenous catheter fixed contralaterally to the jugular vein into an heparinized centrifuge tube at 0 time (before drug administration), and thereafter at 2, 5, 10, 15, 30 min and 1, 2, 4, 6, 8, 12 and 24 h after intravenous administration while blood samples were collected at 0 time (before drug administration), and at 5, 10, 15, 30 min and 1, 2, 4, 6, 8, 12, 24 and 36 h after intramuscular administration of the drug. Plasma was separated by centrifugation at 5,000 RPM for 10 min at room temperature and stored at −40°C until analysis, which usually took place within 24-36 h after collection.

HPLC assay of cefoperazone and pharmacokinetic analysis
Plasma cefoperazone concentration was determined by the high performance liquid chromatography (HPLC) with minor modifications (13). The HPLC system of Laballiance (USA) comprised of a quaternary gradient delivery pump (model AIS 2000) and UV detector (model 500). Chromatographic separation was performed by using reverse phase C18 column (Whatman®, PARTISIL ODS-3 RAC-II; 4.60 X 100 mm ID) at room temperature. The data integration was performed using software “Clarity” (Version 2.4.0.190). The mobile phase was a mixture of 30 mM KH$_2$PO$_4$ buffer (70 %), methanol (30 %) at a pH of 5.0. The mobile phase was filtered by 0.45 µ filters and pumped into column at a flow rate of 1.0 ml/min at ambient temperature. The effluent was monitored at 266 nm wavelength.

Plasma samples (250 µl) were deproteinized by addition of a solution containing 10% perchloric acid, methanol and acetonitrile. This was followed by centrifugation for 5 minutes at 10,000 RPM. The clean supernatant was collected and an appropriate aliquot of 20 µl of this supernatant was injected into the loop of HPLC system through a manual injector.

Calibration curves were prepared using the final dilution in plasma by plotting the area of curve at the ordinate and the drug concentration at the abscissa. The sensitivity of assay method for cefoperazone was 1 µg /ml. The assay was sensitive, reproducible and linearity was observed from 1 to 200 µg /ml. The mean correlation coefficient $(R^2)$ was 0.9995. The Pharmacokinetic parameters were calculated by “PK solution” (version 2.0). “PK Solutions 2.0” relies on the use of non-compartmental method of analysis for the estimation of pharmacokinetic parameters.

\[
\text{Half-life: } t_{\frac{1}{2}} = \frac{0.693}{\lambda_{z}}
\]

\[
\text{AUC (0 - } \infty \text{) and AUMC were calculated by Trapezoidal rule.}
\]

\[
\text{AUC}_{\text{x}} = \text{AUC}_{(0-\text{t})} + \frac{C_{n}}{\lambda_{z}}e
\]
Where, $C_n$ is the last concentration

$$V_{d(\text{area})} = \frac{\text{Dose}}{(\beta \times \text{AUC})}$$

(For Intravenous Injection)

$$V_{d(\text{area})} = \frac{(\text{Dose} \times F)}{(\beta \times \text{AUC})}$$

(For Intramuscular Injection)

$$V_{d(ss)} = \frac{(\text{Dose} \times \text{AUMC})}{(\text{AUC})^2}$$

$$\text{Cl}_{B} = \frac{\text{Dose}}{\text{AUC} \infty}$$

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

$$F = \frac{[\text{Dose} \times \text{AUC (I.M.)}]}{[\text{Dose} \times \text{AUC (I.V.)}] 	imes 100}$$

(For Intramuscular Injection)

**Statistical Analysis**

The cefoperazone concentration in plasma and pharmacokinetic parameters following intravenous and intramuscular route were analysed by Student’s $t$-test using SPSS software (version 12.0.1). Statistical signfiance was considered at $p<0.05$. The pharmacokinetic parameters were expressed in terms of Mean ± Standard Error (S.E.).

**RESULTS**

Following respective intravenous and intramuscular administration, experimental data were found best fitted to the non-compartmental approach. The drug was detected in plasma up to 8 and 12 h following intravenous and intramuscular administration, respectively. Comparative disposition of cefoperazone following single dose intravenous and intramuscular administration in sheep was plotted on semilogarithmic scale (Figure 1).

![Figure 1](image_url)

**Table 1: Pharmacokinetic parameters of cefoperazone after single dose intravenous and intramuscular administration (20 mg/kg) in sheep**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Intravenous (Mean ± S.E., n = 6)</th>
<th>Intramuscular (Mean ± S.E., n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2\alpha}$</td>
<td>H</td>
<td>0.53±0.16</td>
<td>-------</td>
</tr>
<tr>
<td>$t_{1/2\beta}$</td>
<td>H</td>
<td>3.80±0.60</td>
<td>3.32±0.68</td>
</tr>
<tr>
<td>AUC</td>
<td>µg.h/mL</td>
<td>86.43±5.15</td>
<td>60.99±4.27</td>
</tr>
<tr>
<td>$V_{d(ss)}$</td>
<td>L/kg</td>
<td>0.51±0.02</td>
<td>-------</td>
</tr>
<tr>
<td>$\text{Cl}_{B}$</td>
<td>mL/min/kg</td>
<td>5.16±0.32</td>
<td>-------</td>
</tr>
<tr>
<td>MRT</td>
<td>H</td>
<td>3.29±0.57</td>
<td>4.27±0.58</td>
</tr>
<tr>
<td>F</td>
<td>%</td>
<td>-------</td>
<td>71.83±5.96</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>µg/mL</td>
<td>-------</td>
<td>25.67±3.02</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>H</td>
<td>-------</td>
<td>0.5±0.0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Following intravenous administration, the drug was rapidly eliminated ($t_{1/2\beta}$: 3.80±0.60 h) from the body with a clearance rate of 5.16±0.32 mL/min/kg. Following intramuscular administration, the peak plasma drug concentration was 25.67±3.02 µg/mL at 0.5 h and the drug was detected up to 12 h. The drug was rapidly absorbed from the site of injection, widely distributed ($V_{d(\text{area})}$: 1.49±0.32 L/kg) and slowly eliminated from the body ($t_{1/2\beta}$: 3.32±0.68 h; $\text{Cl}_{B}$: 3.30±0.27 mL/min/kg). The bioavailability of cefoperazone was 71.83±5.96 % following intramuscular injection. Various pharmacokinetic parameters calculated from plasma concentrations of cefoperazone after single dose intravenous and intramuscular administrations are summarized in Table 1.
have been reported in cross-bred cattle calves (8). Peak plasma concentration ($C_{\text{max}}$) of 25.67 ± 3.02 µg/mL was observed at 0.5 h following intramuscular injection. However, the lower value of the $C_{\text{max}}$ like 24.5 ± 3.0 µg/mL at 0.45 h in dog (5), 7.98 µg/mL at 0.26 h in horse (11), 9.76 ± 0.25 µg/mL at 0.75 h in cross-bred calves (8) have been reported.

Following intravenous administration, higher value of distribution rate constant ($\alpha$; 2.35 ± 0.28 /h) and low value of elimination rate constant ($\beta$; 0.22 ± 0.04 /h) were observed in the present study. The elimination half life ($t_{1/2}$) of Cefoperazone following intravenous administration in sheep was found to be 3.80 ± 0.60 h, which is higher than the elimination half-life of 2.13 ± 0.47 h reported in unweaned calves (7), 2.05 ± 0.20 h in cross-bred calves (8). The value of elimination half-life of Cefoperazone observed in present study is shorter than the half-life of 5.65 h reported in buffalo calves (9). The elimination half life of Cefoperazone following intramuscular administration in the present study was 3.32 ± 0.68 h. It is longer than the half-life 2.32 ± 0.11 h, 2.28 ± 0.32 h, 2.23 ± 0.21 h and 1.52 ± 0.15 h observed in cross-bred cow calves (8), unweaned cow calves (7), dog (5) and horses (11), respectively.

The total body clearance of Cefoperazone in the present study while following intravenous and intramuscular administration of the drug was 5.16 ± 0.32 and 3.30 ± 0.87 ml/min/kg respectively. The total body clearance following intravenous administration observed in the present study is faster than 1.96, 3.09 and 0.72 ± 0.18 ml/min/kg reported in dog (5), buffalo calves (9) and horses (11), respectively. The total body clearance is slower than 11.5 ± 0.33 ml/min/kg reported in unweaned calves (7) and 8.16 ± 1.60 ml/min/kg reported in unweaned cow calves (7). The result indicates faster clearance of Cefoperazone from the body of sheep following intravenous administration, in comparison to buffalo calves, cows and horses. Elimination half life and total body clearance suggests faster elimination of cefoperazone in sheep.

The mean apparent volume of distribution ($V_{\text{d,area}}$) calculated following intravenous administration were 1.06 ± 0.13 L/kg. This value of $V_{\text{d,area}}$ is similar to the reported values of 1.69 L/kg in unweaned cow calves (7) and 1.3 L/kg in buffalo calves (9). Lower values of $V_{\text{d,area}}$ of 0.23 in dog (5), 0.16 L/kg in sheep (15) and 0.31 ± 0.09 L/kg in horse (11) were reported. The mean apparent volume of distribution ($V_{\text{d,area}}$) calculated following intramuscular administration was 1.49 ± 0.31 L/kg which is in agreement with $V_{\text{d,area}}$ of 1.3 L/kg observed in buffalo calves (9). Very high $V_{\text{d,area}}$ of 4.22 ± 0.35 was also reported in cross-bred cow calves (16). The values of volume of distribution following intravenous administration of Cefoperazone in sheep indicates moderate distribution of drug in the body. However, extensive apparent volume of distribution has been observed following intramuscular administration.

The AUC following intravenous administration was observed to be 86.43 ± 5.15 µg/h/ml which is higher than the value of 37.57 ± 5.55 µg/h/ml observed in horse (11) and 29.0 ± 1.03 µg h/ml observed in cross-bred calves (8). Following intramuscular administration, the value of AUC was 60.99 ± 4.27 µg/h/ml. The value of AUC observed in the present study is higher than the value of 15.70 ± 1.64 µg h/ml reported in horse (11) and 15.70 ± 0.64 µg h/ml reported in cross-bred calves (16). The values of the systemic bioavailability was 71.83 ± 5.96 per cent following intramuscular administration, which is similar to 76.3 in unweaned calves (7) and higher to 48.1 ± 5.33, 42, 41.4 ± 7.1 % reported in cross-bred calves (16), horse (11) and dog (5), respectively. High bioavailability of cefoperazone and maintenance of therapeutic concentration up to 12 h following intramuscular injection suggests that Cefoperazone is most suitable for intramuscular administration for the treatment for systemic bacterial infections in sheep.

Minimum inhibitory concentration for a majority of Cefoperazone sensitive bacteria is in the range of 0.05 to 4.0 µg/ml. MIC$_{90}$ for Gram-positive bacteria like Streplococcus spp. ranges between 0.12-0.25 with mean of 0.185 µg/ml and for most of the Gram-negative bacteria (Escherichia coli, Citrobacter, Klebsiella, Proteus, Neisseria, Morganella and variety of Enterobacter spp.) ranges between 0.005-1 µg/ml (17, 18). Integrating the pooled Cefoperazone pharmacokinetic data generated from the present study with the MIC$_{90}$ range for most of the gram-positive and gram-negative microorganisms, a Cefoperazone dose of 20 mg/kg is sufficient to maintain plasma concentration of the drug above the MIC$_{90}$ when it is administered at 12 h interval following either intravenous or intramuscular administration.

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REFERENCES


