ABSTRACT: The pharmacokinetics of ceftriaxone was studied after intramuscular administration @ 20 mg/kg in adult Mizo local pig (zovawk). The drug concentration in plasma was quantified through High Performance Liquid Chromatography (HPLC) with UV detector. The maximum plasma concentrations (C\text{max}) of 25.92±1.05 µg.ml\textsuperscript{-1}was achieved at 0.5 h (T\text{max}), while the lowest plasma ceftriaxone concentration of 0.22±0.04 µg.ml\textsuperscript{-1} was observed at 24 h following intramuscular administration of ceftriaxone. It has been observed that plasma concentrations of ceftriaxone was maintained up to 24 h (1440 min) during the present investigation following IM administration of ceftriaxone @ 20 mg.kg\textsuperscript{-1} body weight. Pharmacokinetic profile and excellent bioavailability of ceftriaxone indicated that the drug can be used intramuscularly to treat susceptible bacterial infections in pig.

Key words: Ceftriaxone, Pharmacokinetics, Pig.

INTRODUCTION

Ceftriaxone is a member of third generation semi-synthetic cephalosporin preferentially exhibiting more potent activity against aerobic gram-negative than gram-positive bacteria (Brogden and Ward 1988). Ceftriaxone gets distributed in a wide variety of tissues and body fluids such as pleural fluid, peritoneal fluid, bile, bronchial mucosa, myometrium and bone (Papich and Riviere 2010). It crosses not only the inflamed but also the healthy blood-cerebrospinal fluid barrier in horses and man (Richards et al. 1984; Ringger et al. 1996). Favorable kinetic parameters of ceftriaxone are good absorption, high bioavailability and exceptionally long elimination half-life in human beings (Brogden and Ward 1988). The disposition kinetics and dosage schedule of ceftriaxone and related drugs have been determined in goats (Ismail 2005; Sar et al. 2006; Tiwari et al. 2009), sheep (Goudah et al. 2006; Sinha et al. 2015), cattle (Johal and...

Pharmacokinetic data of ceftriaxone after single dose intramuscular administration are lacking in pig. Literature related to pharmacokinetics of ceftriaxone in Mizo local pig are scarcely available. Hence, the present study was planned to evaluate the pharmacokinetics of ceftriaxone in Mizo local pig following single dose intramuscular administration @ 20 mg/kg body weight.

MATERIALS AND METHODS
Experimental animals and drug administration

The experiment was conducted in five healthy female adult Mizo local pig (Sus scrofa) of 7-8 months of age and weighing 20-32.5 kg. The animals were kept at ambient temperature of about 20-27°C with relative humidity ranging from 60-80% with 12 hour light: dark cycle. Water, concentrate feed mixture and green vegetables were provided ad libitum. Animals were kept in homogenous management environment. Weekly health checks of all pigs were carried out in addition to daily observation. Two weeks before the commencement of the
Table 1. Pharmacokinetics parameters of ceftriaxone after single intramuscular administration of ceftriaxone (20 mg.kg⁻¹) in pigs (Mean with SE of 5 replicates).

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Unit</th>
<th>Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vₙ₅</td>
<td>L.kg⁻¹</td>
<td>7.23±2.07</td>
</tr>
<tr>
<td>AUCₘ₈</td>
<td>µg.h.ml⁻¹</td>
<td>47.95±3.11</td>
</tr>
<tr>
<td>Cl</td>
<td>ml.h⁻¹</td>
<td>431.94±9.36</td>
</tr>
<tr>
<td>AUMCₘ₈</td>
<td>µg.h².ml⁻¹</td>
<td>285.08±13.10</td>
</tr>
<tr>
<td>MRT</td>
<td>H</td>
<td>5.69±1.57</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>H</td>
<td>3.94±1.31</td>
</tr>
<tr>
<td>K</td>
<td>h⁻¹</td>
<td>0.21±0.12</td>
</tr>
<tr>
<td>Vₘ₅</td>
<td>L.kg⁻¹</td>
<td>2.35±0.88</td>
</tr>
<tr>
<td>C_max</td>
<td>µg.ml⁻¹</td>
<td>25.92±1.18</td>
</tr>
<tr>
<td>T_max</td>
<td>H</td>
<td>0.5±0</td>
</tr>
<tr>
<td>Ka₁/₂</td>
<td>H</td>
<td>0.35±0.19</td>
</tr>
<tr>
<td>MAT</td>
<td>H</td>
<td>4.07±1.63</td>
</tr>
<tr>
<td>F</td>
<td>%</td>
<td>149.50±0.24</td>
</tr>
</tbody>
</table>

[Vd: Apparent volume of distribution; AUC∞: Total area under curve; AUMC∞: Total area under moment curve; Cl: Total body clearance; MRT: Mean residence time; t₁/₂: Elimination (biological) half-life; K: Apparent overall 1st order elimination Rate constant; Vₘ₅: Steady state volume of distribution; C_max: Maximum plasma concentration; T_max: Time to reach maximum plasma concentration; t₁/₂(Ka): Absorption half-life; MAT: Mean absorption time; F: Bioavailability].

Institutional Animal Ethics Committee was taken for conducting the experimentation.

Collection of Blood Samples

Blood samples (approx. 3.0 ml each) were collected in heparinized test tubes through the anterior vena cava (depicted in Fig. 2) by disposable syringe with 22 gauge size needle at 2.5, 5, 10, 15, 30 min and 1, 2, 4, 8, 12, 24, 36 and 48 h after intramuscular administration in deep gluteal muscle (depicted in Fig.1). Plasma was separated by centrifugation at 3,000 rpm for 10 min at room temperature and stored at -20°C until assayed.

Ceftriaxone Assay

Plasma ceftriaxone concentration was determined by the high performance liquid chromatography (HPLC) method cited by Hakim et al. (1988) and Tiwari et al. (2009). The HPLC system (Waters, U.S.A) consists of isocratic pump (L-7110) with an online degasser (L-7612), interface (D-7000), UV detector (7400), manual injection, chromatography data station software (Millenium) and multi HSM-manager. Chromatographic separation was done using Lichrocart RP-18 column (250 mm X 4 mm) at room temperature. Samples (250 µl) were deproteinized by addition of acetonitrile (500 µl), vortexed for one minute followed by centrifugation for 10 min at 5,000 rpm. A clear supernatant fluid was decanted in a glass insert from which 50 µl was injected into the HPLC system. The mobile phase consisted of a mixture of buffer and acetonitrile (62:38). The buffer was prepared by dissolving 1.78 g of di-sodium hydrogen phosphate dihydrate and 1.0 g of N-acetyl-N, N, N-trimethylammonium bromide in 950 mL of Milli Q water, pH 7.0 was adjusted with orthophosphoric acid. Mobile phase was filtered through 0.45μ Millipore filter.

experiment, all animals were dewormed by oral feeding of fenbendazole (Panacur® bolus, Intervet) @ 7.5 mg.kg⁻¹ body weight. Ceftriaxone (Injection Intacef®, 500 mg; Intas Pharmaceuticals Ltd., Ahmedabad) @ 20 mg.kg⁻¹ body weight was given by intramuscular injection to adult female pigs. A washout period of 3 weeks was observed between treatments. Due approval of the
phase was pumped through column at a flow rate of 1.0 mL/min, at an ambient temperature of 25°C. The elute was monitored at a wavelength of 254 nm.

Standard calibration curve of ceftriaxone
Ceftriaxone (CMS 1334-1G, Himedia Laboratories Pvt. Ltd., Mumbai, India) standards concentration of 0.19, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 µg/ml were prepared by serial dilutions of stock solution (200µg/ml) in drug-free plasma of pig. Calibration curve was prepared for drug concentrations ranging from 0.19 to 50 µg/ml and was used to quantify the drug concentration in samples. The calibration curve was prepared daily and not accepted unless it had a R² value =0.999 and was depicted in Fig. 3. The assay was linear for drug concentrations of 0.19 to 50 µg/ml (R²=0.999). The limit of sensitivity for ceftriaxone in pig plasma was 0.012 µg/ml. The lower limit of quantification of the assay was 0.19 µg/ml.

Pharmacokinetic Parameters
The plasma concentration versus time profile data was subjected to non-compartmental pharmacokinetic analysis using statistical moment approach (Yamaoka et al. 1978; Singh, 1999a and 1999b).

Statistical Analysis
The data were subjected to statistical analysis by employing unpaired ‘t’ test (5% level of significance) using the software SYSTAT VERSION 11.0.1.

RESULTS AND DISCUSSION
Pharmacokinetic analysis revealed that the drug (ceftriaxone) was detected in plasma up to 24 h following intramuscular administration. Disposition of ceftriaxone following single dose intramuscular administration in pig is shown on semi-logarithmic scale in Fig. 4. The therapeutically effective plasma ceftriaxone concentration of 0.22±0.04 µg.ml⁻¹ was maintained up to 24 h following single dose intramuscular administration, which are above the MIC values of some of the ceftriaxone sensitive microbial pathogens. Peak plasma
drug concentration of 25.92±1.05µg.ml⁻¹ was obtained at 0.5 h after intramuscular administration. Pharmacokinetic parameters determined following intramuscular administration of the drug are depicted in Table 1.

In the present study, the mean peak plasma concentration (Cₘₐₓ) of 25.92±1.05 µg.ml⁻¹ was observed at Tₘₐₓ of 0.5± 0 h, following intramuscular administration which was higher than that reported for ceftriaxone in goats. Cₘₐₓ (21.51 ± 0.61 µg.ml⁻¹) was observed at Tₘₐₓ of 0.5 h at the same dose rate (Tiwari et al. 2009). The elimination half-life obtained in the present study after intramuscular administration was 3.94±1.31 h which was considerably shorter than that reported in crossbred calves (6.54±0.87 h) at a dose rate of 10 mg.kg⁻¹ (Johal and Srivastava 1998). Further, the elimination half-life was longer than that reported in dogs (1.17 h) at dose rate of 50 mg.kg⁻¹ (Rebuelto et al. 2002) and in goats (2.03±0.09 h) at the dose rate of 20 mg.kg⁻¹ (Tiwari et al. 2009). The total body clearance obtained in the present study was 7.19±9.36 ml.min.kg⁻¹. A lower clearance value of 4.01±0.3 mL.min.kg⁻¹ was obtained in buffalo calves at the dose rate of 10 mg.kg⁻¹ (Gohil et al. 2009). The mean residence time obtained in the present study was 5.69±1.57 h which was considerably longer than that reported in cow calves (2.29±0.33 h) at 10 mg.kg⁻¹ (Soback and Ziv 1998) and in goats (2.76±0.13 h) at 20 mg.kg⁻¹ (Tiwari et al. 2009). Further, it was shorter than that reported in crossbred calves (9.46±1.2 h) at 10 mg.kg⁻¹ (Johal and Srivastava 1998).

The bioavailability (F) of ceftriaxone following single intramuscular injection @ 20 mg.kg⁻¹ was excellent (149.50±0.24 %) indicating that the drug is slowly and completely absorbed. Other workers have reported 102% bioavailability in dog following single intramuscular injection @ 50 mg.kg⁻¹ (Rebuelto et al. 2002) and 70.2±2.0 % bioavailability in buffalo calves following single intramuscular injection @ 10 mg.kg⁻¹ (Gohil et al. 2009). Patel et al. 2010 reported bioavailability of cefepime more than 100 % in sheep (103.0 ± 8.0 %) following single intramuscular administration.

**CONCLUSION**

From the above study, it may be concluded that ceftriaxone persisted in pig for a longer period with a half life of 3.94±1.31 h and it can penetrate the body fluid widely.

**ACKNOWLEDGEMENT**

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**REFERENCES**


