Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(3): 1825-1828

Received: 24-03-2018
Accepted: 26-04-2018

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Pharmacokinetic of single dose intramuscular administration of cefquinome using microbiological assay in goats

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Abstract
The present study was planned to investigate the disposition kinetics of cefquinome at the dose rate of 2 mg kg^{-1}body weight intramuscularly in healthy marwari male goats at the Livestock Research Station, Kodamdesar, Rajasthan University of Veterinary and Animal Sciences, Bikaner in the month of October, 2016. Plasma concentrations were determined by microbiological assay method using MTCC equivalent (MTCC 1541) of *Micrococcus luteus* (ATCC 9341) as the test organism. Following intramuscular administration of cefquinome, peak plasma concentration (C_{max}) was achieved 5.10 ± 0.51 µg ml^{-1}at 1 h (t_{max}). Absorption half-life ($t_{1/2ka}$), elimination half-life ($t_{1/2\beta}$), area under the plasma concentration time curve (AUC) and volume of distribution (Vd_{area}) were 0.29 ± 0.04 h, 1.48 ± 0.04 h, 14.44 ± 0.82 µg ml^{-1}h and 0.29 ± 0.02 L kg^{-1} respectively. In the present study, the plasma levels above the MIC level were maintained up to 6 h following intramuscular administration of cefquinome so as per the general recommendation that T>MIC should be at least 50% of dosage interval for optimum bactericidal effect, a 12 h dosing interval at the rate of 2 mg kg^{-1} is recommended intramuscularly in goats.

Keywords: Bactericidal, plasma concentration, volume of distribution

1. Introduction
Cefquinome is a fourth generation aminothiazolyl cephalosporin with broad spectrum of activity against Gram-positive and Gram-negative bacteria, developed exclusively for veterinary use including food animals [15]. It has a broad spectrum of antibacterial activity with time dependent bactericidal effect, as shown by β-lactam antibiotics [19]. It has certain advantages over the preceding cephalosporins: i) it possesses extended spectrum of activity, ii) penetration ability into the periplasmic space of Gram-negative bacteria and enhanced binding with penicillin-binding proteins [5], iii) stable against chromosomal, iv) and plasmid-encoded β-1 lactamases that are produced by a majority of clinically important bacteria [13] and v) enhanced bioavailability and improved spectrum of antimicrobial activity compared with the second and third-generation cephalosporins [19]. For the sensible use of an antibiotic in rational dosage, the pharmacokinetic investigations are essential. Keeping in view the significant species variation in pharmacokinetic data of antimicrobials, It has been established that the pharmacokinetics data are to be investigated in animal species domesticated in different climate, in which the drug is to be employed clinically [16,7]. Pharmacokinetics studies offer highly relevant information on the time course of the drugs and their metabolites and facilitate the computation of optimal dosage regimens of drugs to maintain their therapeutic concentration at the biophase [21]. So the current study was planned to determine pharmacokinetic profile of cefquinome in goat following single intramuscular injection by employing microbiological assay method in order to establish adequate dose regimen for potential clinical use in goats diseases caused by susceptible microorganisms.

2. Material and Methods
2.1. Experimental Animals
The study was conducted on five apparently healthy *Marwari* male goats (8- 10 month of age, weight between 25-30 kilograms) at the Livestock Research Station, Kodamdesar, Rajasthan University of Veterinary and Animal Sciences, Bikaner in the month of October, 2016. During the study period they were subjected to clinical examination in order to exclude the possibility of any disease. The animals were housed in separate pens and maintained on concentrate,

green fodder and water *ad libitum*. The experimental protocol and use of animals for conducting the present study has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and submitted to CPCSEA.

2.2. Drugs and Test Organism
Cefquinome sulphate injection (25 mgml^{-1}; Cobactan 2.5%, MSD Healthcare) was procured from local market. In the present study, MTCC equivalent (MTCC 1541) of *Micrococcus luteus* (ATCC 9341) as the test organism used for microbiological assay was procured from institute of Microbial Technology (IMTECH), Chandigarh, India.

2.3. Experimental Protocol
The study was carried out in cross-over design, with a minimum of 15 days of washout period. Cefquinome sulphate injection was given on gluteal muscle at the dose rate of 2 mg kg^{-1} body weight intramuscularly. In the present study, dosage level of cefquinome employed was comparable to the dose of cefquinome used by previous workers in goat [8] and sheep [22]. Blood samples were collected in test tubes containing EDTA, immediately before administration of cefquinome (0 h) and at 0.08, 0.16, 0.33, 0.5, 0.75, 1.0, 1.5, 2, 4, 6, 8, 10, 12 and 24 h after administration of the drug. Collected blood samples were centrifuged at 3000 rpm for 15 min to separate the plasma and plasma samples were stored at −20°C until assayed.

2.4. Drug Bioassay
Concentrations of Cefquinome in plasma samples were estimated by microbiological assay method using MTCC equivalent (MTCC 1541) of *Micrococcus luteus* (ATCC 9341) [3, 9]. By using a punching machine, six equidistant identical (6.0±0.1 mm) wells were punched in the solidified media in petri plates and punched wells were charged with the test samples/cefquinome standard in triplicates. The petri plates were incubated at 30°C for 24 h. In test sample plates, three alternate wells out of six were filled with reference concentration of the drug which gives a clear zone of inhibition of 16-18 mm so as to minimize the plate to plate variation in zones of inhibition. Zones of inhibition were measured by vernier calliper and the mean of triplicate samples was taken and compared with that of reference standard(s) to obtain the concentration of cefquinome in test samples. Minimum sensitivity of the assay method was 0.20 µg ml^{-1}.

2.5. Pharmacokinetic Analysis
The plasma cefquinome concentration time profile of each animal following intramuscular administration of cefquinome used to determine the pharmacokinetic variables describing the absorption, distribution and elimination characteristics of cefquinome in goats. To determine the different disposition kinetic variables, plasma drug concentration–time data were analyzed by employing the compartmental [11, 4] and non-compartmental pharmacokinetic models [24, 11].

2.6. Statistical Analysis
The data in the present studies were subjected to statistical analysis by employing student's 't' test using MS Excel (2007).

3. Result and Discussion
No clinical signs of adverse effects or intolerance were observed to cefquinome IM injection in goats. Plasma cefquinome concentrations at different time intervals following intramuscular injectionina goats is presented as semi logarithmic plot in Fig. 1.Following intramuscular administration of cefquinome at the dose rate of 2 mg kg^{-1} body weight in goats, the plasma concentration of cefquinome was quickly raised well above minimum inhibitory concentration (MIC) against pathogens [1] and found 0.72 ± 0.28 µg ml^{-1} was observed at 0.08 h after drug administration. The peak plasma concentration (C_{max}) was observed 5.10 ± 0.51 µg ml^{-1} at 1.00 h post administration of cefquinome intramuscularly in goats at 2 mg kg^{-1} body weight. In the present study, the higher peak plasma concentration (C_{max}) was observed than in 4.36 ± 0.10 µg ml^{-1} at 0.75 h in sheep [17], 4.84 ± 0.23 µg ml^{-1} at 1.50 h in goat [8], 4.01 ± 0.57 µg ml^{-1} in piglets [12], and 4.83 µg ml^{-1} at 0.43 h in dog [26] administered cefquinome intramuscularly at the same dose. However, the Higher value of the C_{max} like 9.05 ± 0.06 µg ml^{-1} at 0.95 h in rabbits [18]and 9.38 ± 1.69 µg ml^{-1} at 0.38 h in duck [25]. C_{max} achieved earlier in sheep [17], dog [26], rabbit [18] and duck [25]. While later in goats [8] and camel [2].

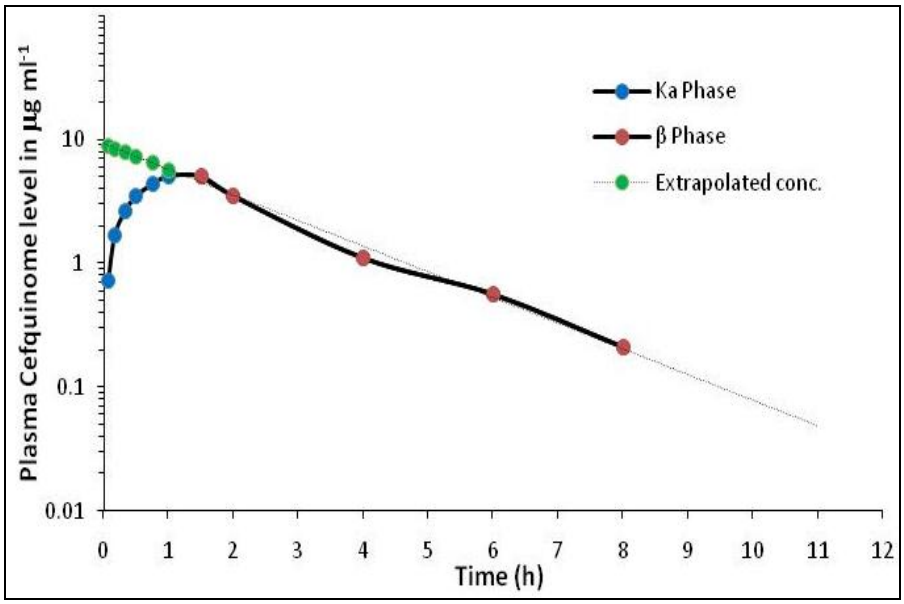

Fig 1: Semilogarithmic plot of mean (n=5) plasma concentration versus time curve of cefquinome given intramuscularly in goats at the dose of 2 mg kg^{-1} body weight.

The points reflecting elimination / β phase (●) and absorption / Ka phase (●) are shown on the curve. The extrapolated plasma concentration values (●) obtained from the method of residuals are also reflected along with the linear regression line going up to zero time intercept.

The disposition kinetics of cefquinome in goats could be explained by one compartment open model considering the plasma concentration versus time semi logarithmic curve. Disposition of cefquinome has been described with one compartment open model in camel [2], goats [8], dog [26] but two- compartment open model in piglets [12], sheep [20] and duck [25].Various pharmacokinetic determinants that describe the absorption and elimination pattern of cefquinome after intramuscular administration were calculated and are presented in Table 1.

Table 1: Pharmacokinetic determinants of cefquinome in goats following a single intramuscular dose rate of 2 mg kg^{-1} body weight employing compartmental model.

Parameter	Unit	Mean ± S.E.
A'	µg ml^{-1}	10.33 ± 1.22
K_a	h^{-1}	2.67 ± 0.52
$t_{1/2ka}$	H	0.29 ± 0.04
B	µg ml^{-1}	9.01 ± 0.97
B	h^{-1}	0.47 ± 0.01
$t_{1/2\beta}$	H	1.48 ± 0.04
$C_{max(obs)}$	µg ml^{-1}	5.39 ± 0.38
$t_{max(obs)}$	H	1.15 ± 0.15
AUC	µg $ml^{-1}h$	14.44 ± 0.82
$AUC_{0-\infty}$	µg $ml^{-1}h$	14.89 ± 0.82
AUMC	µg $ml^{-1}h^2$	38.19 ± 2.45
MRT	H	2.64 ± 0.07
Vd_{area}	L kg^{-1}	0.29 ± 0.02
Cl'	ml $kg^{-1}h^{-1}$	140.33 ± 8.09

The absorption half-life ($t_{½Ka}$) of cefquinome after intramuscular administration in goat was found to be 0.29 ± 0.04 h, suggesting rapid absorption of cefquinome from the injection site. Comparable has been reported 0.28 ± 0.02 h in rabbit [18]. Longer absorption half-life ($t_{½Ka}$) values of cefquinome have been observed 0.61 ± 0.10 h in Sheep [17], 0.64 ± 0.23 h in goat [8] and 4.35 ± 0.27 h in camel [2]. While shorter absorption half-life ($t_{½Ka}$) was reported 0.14 h in beagle dogs [26].

The elimination half-life ($t_{1/2\beta}$) expresses the overall rate of elimination of a drug and allows prediction of drug accumulation. Compartmental analysis of pharmacokinetic profile of cefquinome after intramuscular administration in goat revealed the elimination half-life ($t_{½\beta}$) of 1.48 ± 0.04 h in the present study. It is comparable to elimination half-life ($t_{½\beta}$) value reported 1.79 ± 0.13 h in ducks [25]. Higher elimination half-life ($t_{½\beta}$) values have been reported 12.29 ± 2.62 h in sheep [17], 5.86 ± 0.29 h in goat [8], 4.36 ± 2.35 h in piglet [12] and 10.24 ± 0.8 h in camel [2].

The area under plasma concentration time curve (AUC) is an important parameter used to calculate clearance, volume of distribution and bioavailability of drugs in pharmacokinetic studies. The average value of area under the curve (AUC) after intramuscular administration of cefquinome in goats in the present study was 14.44 ± 0.82 µg ml^{-1} h which is almost comparable to 16.65 ± 0.57 µg ml^{-1} h in sheep [17], 19.82 ± 2.07 µg ml^{-1} h in goats [8] and 20.37 ± 1.1 µg ml^{-1} h camel [2]. But the values of AUC were found higher 38.79 ± 1.24 µg ml^{-1} h in chickens [10] and 43.26 ± 0.69 µg ml^{-1} h in rabbits [18]. Lower value of AUC was reported 8.24 µg ml^{-1} h in beagle dogs [26] and 5.13 ± 1.06 µg ml^{-1} h in chickens [23].

The value of area under moment curve (AUMC) after intramuscular administration of cefquinome in goats in the present study was found to be 38.19 ± 2.45 µg ml^{-1} h^2. Higher AUMC values have been reported 157.05 ± 37.93 µg ml^{-1} h^2 in sheep [17], 155.85 ± 9.70 µg ml^{-1} h^2 in goats [8] and 182.75 ± 5.35 µg ml^{-1} h^2 in rabbits [18].

The time required for an intact drug molecule to transit through body is termed as mean residence time (MRT). The mean residence time (MRT) after intramuscular administration of cefquinome in present study was found to be 2.64 ± 0.07 h. However, higher values of mean residence time (MRT) 9.14 ± 1.83 h in sheep [17], 8.08 ± 0.50 h in goats [8], 16.74 in camel [2] and 4.12 ± 0.05 h in rabbits [18] were reported.

The mean apparent volume of distribution (Vd_{area}) calculated following single dose intramuscular administration of cefquinome (2 mg kg^{-1}) in goats was 0.29 ± 0.02 L kg^{-1}. This value of $Vd_{(area)}$ for goats in present study was lower than the reported values of 0.34 L kg^{-1} in pigs [14]. The result indicates lower distribution of cefquinome in to various body fluids and tissues of goats.

Clearance of a drug indicates the biological fluids (plasma/blood) from which the drug has to be removed per unit of time (Cl_B) to account for its elimination. Total body clearance is a measurement of the ability of body to eliminate drug and represents the sum of different clearance processes of the body e.g hepatic biotrasformation and renal excretion etc. In some pharmacokinetics trials, the bioavailability of the studied drug is not known and the apparent clearance (Cl') reflects the drug clearance does not take in to account the bioavailability of the drug [6]. The apparent clearance of cefquinome in present study following intramuscular administration of drug was 140.33 ± 8.09 L h^{-1} kg^{-1}.

4. Conclusion

In the present study, the plasma levels above the MIC level were maintained up to 6 h following intramuscular administration of cefquinome so as per the general recommendation that for optimum bactericidal effect, T>MIC should be at least 50% of dosage interval, a 12 h dosing interval at the rate of 2 mg kg^{-1} is recommended intramuscularly in goats.The dose regimen recommended in the present study may be considered for clinical use in goats after establishing PD studies and potential clinical testing of cefquinome in this species.

5. Acknowledgement
Author would like to thank Incharge, Livestock Research Station, Kodamdesar, Rajasthan University of Veterinary and Animal science, Bikaner and Principal Investigator, AIRCP on genetic improvement of marwari goats in farmer's flocks, CVAS, Bikaner for their support during the research work by providing physical facility and animals to carry out this work.

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