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Citation	Japanese Journal of Veterinary Research, 64(Supplement 2), S109-S115
Issue Date	2016-04
Doc URL	https://hdl.handle.net/2115/62018
Type	departmental bulletin paper
File Information	S109-115 Eslam Hame.pdf

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Japanese Journal of Veterinary Research 64(Supplement 2): S109-115, 2016

Pharmacokinetics of cefquinome following multiple doses intramuscular administration in goats using HPLC

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Abstract

Pharmacokinetics of cefquinome was investigated in goats following multiple IM doses (2mg/Kg) for 3 successive days using HPLC. The plasma concentration time data was best fitted by two compartment model demonstrating the presence of absorption, distribution and elimination phases. The maximum plasma concentrations (C_{max}) following multiple IM administration were 3.66±0.03, 4.46±0.12 and 5.16±0.14 attained at Tmax of 2 hours and declined to (C_{min}) 0.5±0.04 , 0.7±0.03 and 0.86±0.04 μg/ml at 24 h post drug administration in the first , second and third days respectively. Cefquinome was eliminated with half-life values ($t_{o.5\ el}$) of 6.13±0.54, 7.26±0.31 and 7.52±0.65 h in the first, second and third days post IM administration respectively. Cefquinome had a slight cumulative effect following repeated intramuscular administration and the repeated intramuscular injection of cefquinome at a dose of 2mg/kg with 24 h interval met pharmacokinetic–pharmacodynamic criteria predicting a successful therapy for susceptible bacteria with MIC ≤ 0.39 μg /mL.

Key words: Cefquinome, multiple doses, pharmacokinetics, goats.

Introduction

Cefquinome is a fourth generation cephalosporin approved and used exclusively in veterinary medicine for several animal species in many countries worldwide[1,7)]. Cefquinome has a broad-spectrum in vivo and in vitro activity against gram-positive and gram- negative bacterial species with great resistance against β-lactamase[18)]. The main difference between cefquinome and the earlier cephalosporins is the addition of zwitter- ionic molecular structure to the b-lactam nucleus which enhances faster penetration of the outer plasma membrane of gram-negative bacteria and improve binding with penicillin binding protein[3)]. Clinically, cefquinome is approved firstly for treatment of respiratory tract diseases, acute mastitis and digital dermatitis in cattle then extended for treatment of respiratory diseases, metritis and mastitis in pig. Later on it is approved for use in horse[5-7)]. Pharmacokinetics of cefquinome following a single dose administration were determined in camels[2)], goats[10)], pigs[14)], piglets[17)], calves[9)], chickens[11)], ducks[33)], rabbits[22)], rats[21)] and fishs[23)]. Cefquinome pharmacokinetic following repeated IM dose was studied in sheep and goats using microbiological assay[12)]. The aim of this work is to investigate pharmacokinetics of cefquinome following multiple dosage regimens in goat using high performance liquid chromatography (HPLC).

Materials and Methods

Drugs and chemicals: Cefquinome standard and cefquinome as 2.5% cefquinome suspension with a commercial name (Cobactan® 2.5%) were provided from Intervet International Company, Cairo, Egypt. Acetonitrile and water were HPLC grade and obtained from Lab scan chemical industries, Poland. Tri flouroacetic acid (TFA) was highly purified > 99% purchased from Merck-Schuchardt, Germany.

Animals: Five clinically healthy non lactating Baladi goats weighing (15-18 kg) and aged 1 year were used in this work. Goats were considered healthy before the study on the basis of findings of physical parameters as temperature, pulse, respiration, appetite, faecal consistency and mentation. The animals were housed in hygienic stable and maintained on concentrate and green feed with free access to water. None of animals was treated with any medication 45 days prior to the study. The study was approved by the Animal Use Ethical Committee at Faculty of Veterinary Medicine, Zagazig University, Egypt.

Experimental design: Each goat was injected intramuscularly with a dose of 2mg/kg cefquinome (cobactan 2.5%) in the thigh muscle for three successive days with fixed dosing interval (24h.). Two millilitres(2ml) blood samples were collected from jugular vein of each goat into heparinized tube just before administration of the first dose and at 10 , 20 , 30 and 45 minutes , 1,2,4,6,8,10,12 and 24 hour after each injection. Samples were centrifuged at 3000g for 15 minute to collect plasma and stored at -20 °C until analysis.

Analytical assay: Plasma concentrations of Cefquinome were quantified using revered phase HPLC following liquid extraction by the method previously described [30]. The Surveyor HPLC system (thermo scientific Co. USA) equipped with auto sampler ,vacuum degasser , quaternary pump , photodiode array (PDA) detector and connected to PC (Dell) with chormoQuest 5.0 software for instruments control and data analysis. The separation was done on hypersil gold C 18 (5μm, 150x4.6 mm) column (thermo scientific Co. USA). The mobile phase was acetonitrile: TFA 0.1% at ratio of 50:50 with isocratic method and flow rate of 1 ml/min. 20 ul of prepared sample was injected and monitored at a wave length of 268 nm. Retention time of cefquinome was 1.8 minute.

Method validation: Analytical method was validated in house for precision, linearity, accuracy (recovery %), LOD and LOQ.

Pharmacokinetic analysis: Pharmacokinetic model was determined by visual examination of individual concentration–time curves and by application of Akaike's Information Criterion (AIC)[32]. pharmacokinetic parameters were computed according to standard equations[13] using Microsoft Excel 2010. The peak plasma concentration (Cmax) and the time to Cmax (Tmax) were taken from the plot of plasma concentration- time curves. Area under concentration- time curve (AUC_{0-24}) and Area under the first moment ($AUMC_{0-24}$) were calculated by the trapezoidal rule. The results were statistically analyzed using student "t" test and were expressed as mean and standard error[25].

Results

Clinically, no adverse effects were observed after administration of cefquinome intramuscularly for 3 consecutive days in goats.

A linear relationship existed in the calibration curve of cefquinome over the range of 0.3– 12.5 μg / ml, which yielded a correlation coefficient exceeding 0.99. The intraday and interday precision for cefquinome was 1.56% and 1.95 % respectively and the accuracy of spiked plasma samples were 96-98 %. LOD and LOQ were 0.01 and 0.05 μg / ml respectively. Liquid chromatograms of cequinome standard (12.5 μg / ml) and chromatogram of goat plasma sample at 2 h. after IM administration showed in fig. (1 A, B)

Following intramuscular administration of cefquinome, the plasma concentration time

data was best fitted by two compartment model demonstrating the presence of absorption, distribution and elimination phases. The mean plasma concentration versus time following intramuscular administration of cefquinome once daily for 3 successive days illustrated in fig (2). Estimated Pharmacokinetics parameters presented in table (1)The maximum plasma concentrations (C_{max}) following multiple IM administration were 3.66±0.03, 4.46±0.12 and 5.16±0.14 μg attained at T_{max} of 2 hours and declined to(C_{min}) 0.5±0.04 , 0.7±0.03 and 0.86±0.04 μg/ml at 24 h post drug administration in the first , second and third days respectively. Cefquinome was eliminated with half-life value ($t_{0.5\ el}$) of 6.13±0.54, 7.26±0.31 and 7.52±0.65 h in the first, second and third days post IM administration respectively.

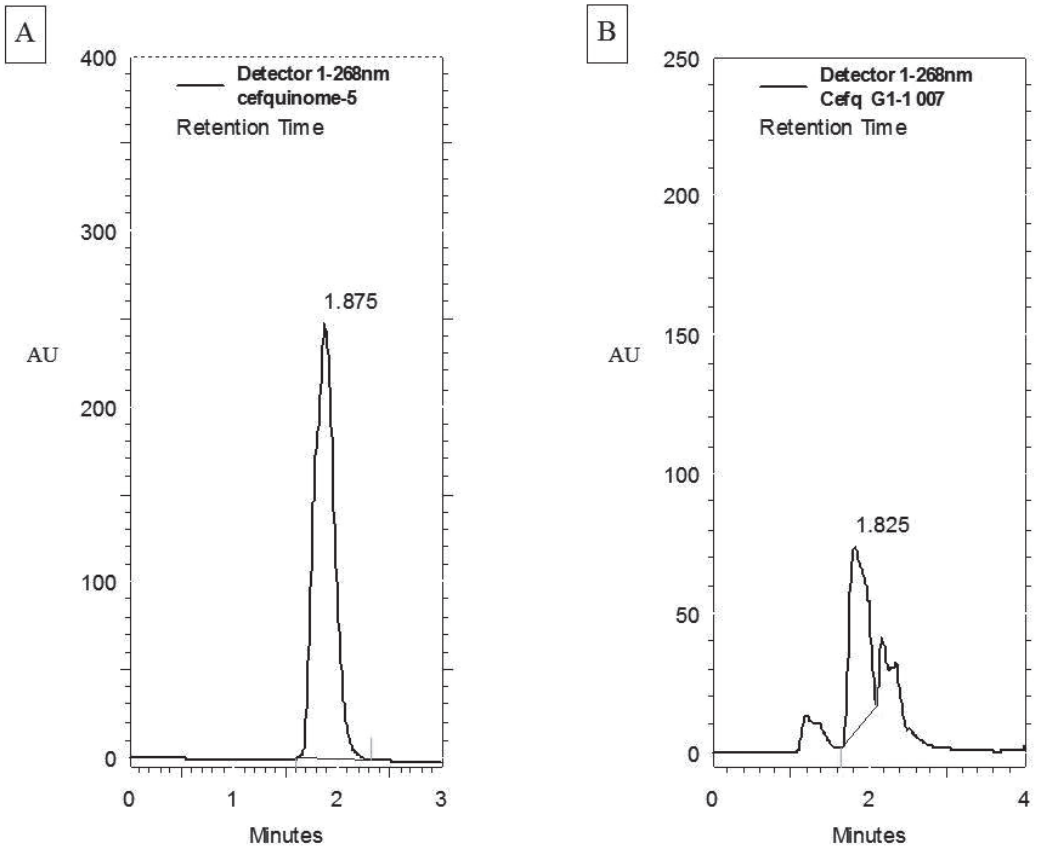

Fig. (1) Representative Liquid Chromatogarms of 20 μl injection of A) cefquinome standard 12.5 μg /ml. B) cefquinom extract from goat plasma 2 hours post administration of a single intramuscular dose (2 mg/Kg).

Table 1. Pharmacokinetic parameters of cefquinome (2 mg/kg) after multiple intramuscular dministration in goats.

parameter	Unit	first day	second day	third day
K_{ab}	h^{-1}	1.13±0.12	0.93±0.08	0.86±0.05
$t_{1/2a}$	h	0.62±0.08	0.75±0.01	0.81±0.02
$t_{1/2\alpha}$	h	0.60±0.04	0.73±0.06	0.77±0.06
K_{el}	h^{-1}	1.11±0.01	0.09±0.01	0.09±0.00
$t_{1/2\beta}$	h	6.13±0.54	7.26±0.31	7.52±0.65
C_{max}	μg/ml	3.66±0.03	4.46±0.12	5.16±0.14
T_{max}	h	2±0.00	2±0.00	2±0.00
AUC $_{0-24}$	μg.h/ml	47±3.5	54±3.7	65±5.22
AUC $_{0-\infty}$	μg.h/ml	52±4.46	61±4.28	75±0.06
AUMC $_{0-24}$	$\mu g.h^2$/ml	396±22	447±41.00	561±0.07
AUMC $_{0-\infty}$	$\mu g.h^2$/ml	542±40	682±71	886±73
MRT	h	10.5±0.9	11.5±0.8	11.9±1.2

K_{ab}, absorption rate constant ; $t_{1/2a}$, absorption half-life; $t_{1/2\alpha}$, distribution half-life; K_{el} , elimination rate constant ; $t_{1/2\beta}$, elimination half-life; AUC,area under concentration–time curve; AUMC,area under the first moment curve; MRT, mean residence time; Cmax, maximum drug concentration; Tmax, time to Cmax

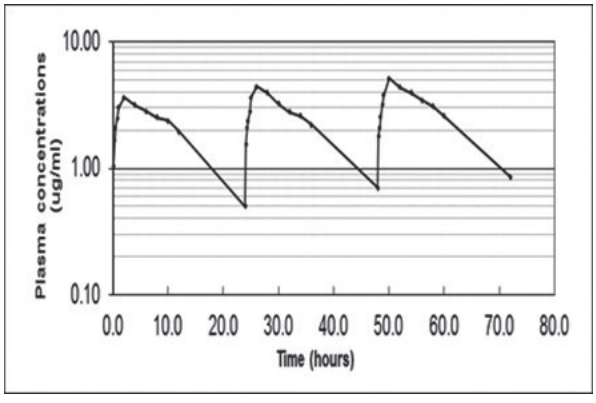

Fig (2) Semilogarithmic plot of cefquinome plasma concentration vs. time after multiple IM administration (2mg/kg) in goats once daily for 3 successive days.

Discussion

In the present study, plasma concentration of cefquinome was determined by HPLC method. HPLC is a Precise, sensitive and well-established technique for measuring solutes in the biological fluids and tissues of laboratory animals and mammals, it is ideal for bio analytical assays[16].

The disposition of cefquinome following intramuscular administration in goat was best described by a two-compartment open model which was similar to that reported in goats and sheep[12] , sheep[30], piglets[17] , ducks[33] , rabbits[22] and fish[23]. However, a one compartment open model was shown to provide the best fit for cefquinome plasma concentration-time data in goat[10] and camels[2].

Following repeated IM administration the absorption half-lives of cefquinome in goats were 0.62±0.08, 0.75±0.01 and 0.81±0.02 h in the 1st, 2nd and 3rd days post administration respectively. This finding was in agreement with 0.64 h reported in goat[10], 0.6 h reported in one year old sheep[27], 0.76 h and 0.73 h in sheep and goat[12]. However, a shorter absorption half-life of the drug has been reported in ducks 0.12 h[33] and chicken 0.17 h[11] after IM administration indicating longer duration for the drug to reach systemic circulation from site of administration and hence slower onset of pharmacological action in goats[10].

The distribution half-lives of cefquinome following repeated IM administration in goats were 0.60±0.04, 0.73±0.06 and 0.77±0.06 h in the 1st, 2nd and 3rd days post administration respectively indicating long duration of time for the drug to distribute and reach different tissues. This may be explained by the hydrophilic nature, low fat

solubility and low pKa values of 2.51 or 2.91 of this compound which might be also the major reason for the limited distribution of cefquinome to the tissues[5].

Cefquinome showed long elimination half-lives (0.5 β) after repeated intramuscular injection in goats 6.13±0.54, 7.26±0.31 and 7.52 ± 0.89 h in the 1st , 2nd and 3rd days post administration respectively. Prolonged elimination half-life has been reported for sheep and goat[12]. However, a shorter elimination half-life has been reported in goats 5.86 h[10] and sheep 2.41 h[27] after intramuscular injection. Such differences are common and frequently related to interspecies variation, assay methods used and the formulation of the drug used[15]. The mean residence time (MRT) of cefquinome was 11.9±1.2 h which was in agreement with 15.16 h in goats[12] and was consistent with 16.74 h recorded in camels[2]. The longer $T_{0.5\beta}$ and MRT of cefquinome in the present study indicated long persistence of the drug.

Repeated drug administration involves administration of the new doses before the previous dose is completely eliminated resulting in drug accumulation until the steady state is achieved. The slight increase in serum concentrations of cefquinome following the 2nd and 3rd doses compared to the first dose indicated the accumulation of cefquinome in blood following intramuscular administration for three successive days with fixed dosing interval (24 h) in goats. These results supported that previously obtained in sheep and goat following repeated IM administration of cefquinome[12].

Overall efficacy of antibacterial agent is related to AUC, and the $AUC_{0\text{-}24}$ /MIC_{90} is the most important predictor that determine the efficacy. A second predictor of efficacy for concentration dependent antibacterial agent is the ratio C_{max}/MIC_{90}. Both numerical values used as pharmacokinetic-pharmacodynamics (PK/PD) surrogate markers for measuring efficacy of β-lactam, quinolones and aminoglycosides only[28,29,31]. Considering the reported MIC_{90s} of cefquinome (0.06–0.39 g/mL) for Escherichia *coli, Pasteurella multocida*, and *Streptococcus agalactiae* [4,8,18,19,20.24,26], the repeated intramuscular injection of cefquinome for 3 successive days at a dose of 2mg/kg is sufficient to maintain serum concentration above MIC_{90} for sensitive susceptible pathogens and meets pharmacokinetic–pharmacodynamic criteria predicting a successful therapy for susceptible bacteria with MIC ≤ 0.39 μg/mL.

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