DISPOSITION KINETICS OF CEFQUINOME IN HEALTHY RABBITS FOLLOWING INTRAMUSCULAR AND ORAL ADMINISTRATION

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ABSTRACT

The target of the present study was to investigate the serum disposition kinetics of cefquinome, a fourth generation cephalosporin, in healthy rabbits (n = 8) following a single intramuscular (IM) injection and single oral (PO) administration at a dose rate of 10 mg/kg b.wt, using a parallel design single dose study. Blood samples were collected at appropriate times during 24 hrs administration interval. Serum samples were analyzed using High Performance Liquid Chromatography-tandem mass spectrometry (HPLC-MS/MS) method. The serum cefquinome disposition kinetic was best fitted to a two-compartment open model after IM and PO dosage. Following intramuscular and oral administration, the major pharmacokinetic parameters (mean ± SD), elimination half-lives (t1/2el) were 2.60 ± 0.04 and 2.32 ± 0.06 hrs, respectively. Values of area under the curve (AUC) were 43.26± 0.69 and 34.69 ± 1.03 µg.h/ml, respectively. Mean residence times (MRT) were 4.12 ± 0.05 and 3.72 ± 0.07 h, respectively. Peaks of cefquinome concentrations (Cmax) were 9.05 ± 0.06 and 8.12 ± 0.11 µg/ml attained at 0.95 ± 0.04 and 1.01 ± 0.08 h (Tmax), respectively. In conclusion the pharmacokinetic profile of cefquinome administered to rabbits by intramuscular and oral routes demonstrate good absorption and long elimination half-life.

KEYWORDS: Cefquinome; Rabbits; Disposition kinetics; Intramuscular; Oral.

INTRODUCTION

Rabbits are quite prone to respiratory diseases. The bacteria most often involved in these complications include Pasteurella multocida and Staphylococcus aureus. However, there
are few antibiotics that can provide a safe and effective therapy for such conditions particularly those caused by resistant strains.

Cephalosporins are among the most widely used group of antibacterial agents in veterinary and human medicine for preventing and treating bacterial infections.\[^4\] Cephalosporins are described as $\beta$-lactam antibiotic drugs, based on their common structural feature, containing the $\beta$-lactam ring. A major advantage of the $\beta$-lactam antibiotics is high degree of safety in the target animal.\[^5\] Cefquinome, an aminothiazolyl cephalosporin, is the fourth-generation cephalosporin antibiotic, which has been developed solely for veterinary use. That drug is bactericidal and acts via binding to penicillin-binding proteins of Gram bacteria to inhibit the cross-linking of peptidoglycan, thereby interfering with cell wall synthesis.\[^6\] Cefquinome shows potent antibacterial activity against a broad spectrum of bacterial species, such as a large number of Gram–positive bacteria, some Gram–negative bacteria, *Vibrio*, *Spirochete*, and *Mycoplasma*.\[^7\]–[^9\] Fourth-generation cephalosporins exhibited marked resistance to $\beta$-lactamases and increased outer membrane permeability, when compared with third-generation cephalosporins.\[^10\] In veterinary medicine, cefquinome is approved and used for several animal species in many countries worldwide.\[^11\] Cefquinome has been extensively used for treatment of cattle and pig against bacterial infections of respiratory tract and the udder.\[^12\]

Cefquinome is bactericidal via a time-dependent mechanism; the pharmacokinetic variable related to the efficacy of the drug is the amount of time that the serum drug concentration exceeds the MIC for a pathogen. The time that the serum concentration of a drug is greater than the MIC for a pathogen should be 40% to 60% of the interdose interval. For such drugs, circulating concentrations $>$ the MIC do not result in increased efficacy against bacteria.\[^13\]–[^16\]

The pharmacokinetics of cefquinome has been extensively investigated in various animal species, pigs, dogs, cattle, sheep, goats, chicken, buffalo calves and ducks.\[^17\]–[^25\] In those studies, favorable pharmacokinetic features of cefquinome, such as good absorption, high bioavailability, low protein binding, and primarily eliminated unchanged via the kidney are found. However, there are few reports available on its disposition in rabbits.\[^26\],[^27\] However, in both of them there is no report on pharmacokinetics of cefquinome after the oral route has been reported yet, to the authors’ knowledge, while there are a little on the disposition of cephalosporins in rabbits.\[^28\]–[^30\] Therefore, the aim of the present study was to investigate the
pharmacokinetics of cefquinome (10 mg/kg) in healthy rabbits following single oral (PO) and intramuscular (IM) administration. Sympathy the pharmacokinetics of cefquinome may allow for better design of future studies to evaluate the medicine-based effectiveness in rabbits and its allometric characteristics.

MATERIAL AND METHODS

Drugs and chemicals

Cefquinome (Cobactan® 2.5%), Cefquinome was obtained from Intervet International Company, Cairo, Egypt. The solvents (Baker Inc., Phillipsburg, NJ, USA) used during the chromatographic analysis of the drug were of HPLC grade.

Animals

Eight New Zealand white rabbits of both sexes, 10 –12 months old and weighing 2.250–3.200 kg, were obtained two weeks before the start of the study. The rabbits were housed individually in cages under a 12-h light/dark cycle and fed good quality hay (alfalfa) and a pelleted feed concentrate (fiber 18%, protein 14%, calcium >1 and fat 2%) with free access to water. The room temperature and relative humidity were maintained at 20 and 22°C, and between 30 and 60%, respectively. The animals were allowed to acclimatize and did not receive any drug treatment for at least 15 days preceding the study. The study was reviewed and approved by the Institutional Animal Care and Use Committee at Faculty of Veterinary Medicine, Cairo University, Egypt.

Drug administration

The study was performed as a parallel design with a single treatment period to avoid the physiological changes in young and rapidly growing animals which may alter the pharmacokinetics of the drug between the first and second period as in the case of a cross-over design. Four rabbits were given a single oral administration of cefquinome 10 mg/kg b.wt by rabbit’s stomach tube and a syringe. The other four rabbits were injected intramuscularly into the left semimembranous muscle with the drug at the same dose. Blood samples (0.5 ml each) were taken via indwelling catheter into Vacationers (Becton Dickinson vacationer Systems, Rutherford, NJ, USA), from the right ear vein at 0 (blank sample), 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hr after treatment. All the blood samples were centrifuged at 3000 rpm for 10 min to separate serum. Serum samples were frozen at –20 °C until analyzed.
Analytical method
Serum concentrations of cefquinome were measured using a modified High Performance Liquid Chromatography-tandem mass spectrometry (HPLC-MS/MS) method previously reported by Zhang et al.[31]

Calibration curve
The calibration curves of serum were prepared with seven different concentrations between 0.01 and 10 μg/ml using blank rabbits serum. A calibration curve was obtained by plotting the cefquinome peak areas versus known concentrations. The equation was calculated by the least-squares method using linear regression. The minimum quantitative limit (LOQ) of the assay was 0.01 μg/ml. The standard curve of cefquinome in rabbit’s serum was linear between 0.01 and 10 μg/ml, the value of the correlation coefficient (r) was > 0.99. The peak area ratios of an unknown specimen (peak area of cefquinome /peak area of internal standard) were compared with that of the standard.

Validation of the assay method
The precision and accuracy of the LC/MS/MS assay were determined via repeat analysis (n=12 analyses) of the serum standard samples containing various concentrations of cefquinome. Percentage recovery of cefquinome was determined by comparing the peak areas of blank samples spiked with different amounts of drug and treated as any samples, with the peak areas of the same standards prepared in phosphate buffer (n=6). Intra-assay variations were determined by measuring six replicates (n=6) of three standard samples used for calibration curves. The intra-assay variation coefficient was < 4.3. Inter-assay precisions were determined by assaying the three standard samples on three separate days. The inter-assay variation coefficient was< 4.7. Recovery of cefquinome from plasma was found to be 97 %.

Pharmacokinetic analysis
A computerized curve-stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration–time curves for each individual animal following the administration of cefquinome. For oral and intramuscular administration, the appropriate pharmacokinetic model was determined by the visual examination of individual concentration–time curves and by application of Akaike’s Information Criterion (AIC).[32]
Each individual curve of cefquinome over time was analyzed in order to determine the peak concentration (C$_{\text{max}}$) and the time to peak concentration (T$_{\text{max}}$). The program also calculated the non-compartmental parameters using the statistical moment theory.$^{[32]}$ The terminal elimination half-life (t$_{\text{1/2el}}$) and absorption half-life (t$_{\text{1/2ab}}$) were calculated were calculated using standard equations.$^{[33]}$ The area under serum concentration–time curve (AUC) and area under the first moment curve (AUMC) were calculated by the method of trapezoids, and extrapolation to infinity was performed.

**Statistical analysis**

The statistical analysis was performed using the SPSS® 17.1 software package (SAS, Cary, NC, USA). Results are presented as mean ± SD. The non-parametric Wilcoxon test was used to compare the parameters obtained after PO and IM. Means were considered significantly different at P< 0.05.

**RESULTS**

Clinical examination of all animals before and after each trial did not reveal any abnormalities. None of the rabbits had treatment related adverse effects during the study. Akaike's Information Criterion test indicated that a two-compartment model best represented the serum concentration versus time data after oral and intramuscular administration of cefquinome in rabbits.

![Semilogarithmic plot depicting the time-course of cefquinome (Mean ± SD) in serum of healthy rabbits after a single oral (●) and intramuscular (■) administration of 10 mg/kg body Weight. (n=4 rabbits).](image)

Figure 1. Semilogarithmic plot depicting the time-course of cefquinome (Mean ± SD) in serum of healthy rabbits after a single oral (●) and intramuscular (■) administration of 10 mg/kg body Weight. (n=4 rabbits).
The Mean ± SD serum concentration-time profiles of cefquinome following single IM and PO administrations of 10 mg/kg b.wt are illustrated in Figure 1. Mean ± SD values of pharmacokinetic parameters estimated from the curve fitting are recorded in Table 1. Statistical analysis of the serum pharmacokinetic parameters revealed significant differences in the elimination rate constant, elimination half-life, area under the curve (AUC), AUMC, MRT and $C_{\text{max}}$ between IM and PO administration.

Table1. Pharmacokinetic parameters of cefquinome following a single oral and intramuscular administration (10 mg/kg b.wt.) in healthy rabbits. (Mean ±SD, n=4).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>PO</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B$</td>
<td>$\mu g/ml$</td>
<td>12.04±0.65</td>
<td>13.13±0.21*</td>
</tr>
<tr>
<td>$A$</td>
<td>$\mu g/ml$</td>
<td>13.96±0.44</td>
<td>14.92±0.92</td>
</tr>
<tr>
<td>$k_{ab}$</td>
<td>$h^{-1}$</td>
<td>2.75±0.55</td>
<td>2.49±0.17</td>
</tr>
<tr>
<td>$t_{1/2ab}$</td>
<td>$h$</td>
<td>0.26±0.05</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td>$k_{el}$</td>
<td>$h^{-1}$</td>
<td>0.295±0.01</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td>$t_{1/2el}$</td>
<td>$h$</td>
<td>2.32±0.06</td>
<td>2.6±0.04***</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$</td>
<td>$\mu g.h/ml$</td>
<td>34.69±1.03</td>
<td>43.26±0.69***</td>
</tr>
<tr>
<td>AUMC</td>
<td>$\mu g.h^2/ml$</td>
<td>133.01±6.15</td>
<td>182.75±5.35***</td>
</tr>
<tr>
<td>MRT</td>
<td>$h$</td>
<td>3.72±0.07</td>
<td>4.12±0.05***</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>$\mu g/ml$</td>
<td>8.12±0.11</td>
<td>9.05±0.06***</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>$h$</td>
<td>1.01±0.08</td>
<td>0.97±0.04</td>
</tr>
</tbody>
</table>

A = Zero-time intercept of the distribution; B = Zero-time intercept of decline in serum concentration of drug; $k_{el}$: elimination rate constant; $k_{ab}$: absorption rate constant; $t_{1/2ab}$: absorption half-life; $t_{1/2el}$: elimination half-life; AUC: area under the curve from zero to infinity by the trapezoidal integral; AUMC: area under moment curve by the trapezoidal integral; $C_{\text{max}}$: maximum plasma concentration; MRT: mean residence time; $T_{\text{max}}$: time to peak concentration; Values after IM administration were significantly different from corresponding values following PO administration. *P<0.05.

Following oral administration, cefquinome was determined in a concentration of (3.45±0.11 $\mu g/ml$) 15 min. post administration which increased gradually till reached its maximum serum level (7.98±0.20 $\mu g/ml$) at 1 hour post administration. The drug is still detected until 12 hours after administration (0.08±0.01 $\mu g/ml$). No cefquinome concentration could be detected thereafter.

Following intramuscular administration, cefquinome was determined in a concentration of (5.73±0.16 $\mu g/ml$) 15 min. post administration which increased gradually till reached its
maximum serum level (8.92±0.11 µg/ml) at 1 hour post administration. The drug is still detected until 12 hours after administration (0.29±0.02 µg/ml). No cefquinome concentration could be detected thereafter.

**DISCUSSION**

No clinical abnormalities of all rabbits were detected during the study. No local signs of pain or soft tissue swelling at injection sites or systemic adverse reactions to cefquinome were detected in rabbits after intramuscular or oral administration. The present investigation revealed that serum cefquinome concentrations versus time decreased in a bi-exponential manner following intramuscular and oral administration. This finding was previously reported for cefquinome in ducks,\(^{[24]}\) chickens,\(^{[21]}\) and is in agreement with other studies of cephalosporins in rabbits\(^{[28,30]}\) but not agree with that reported in goats and camels on using cefquinome.\(^{[22,34]}\)

Following single intramuscular and oral administration of cefquinome at a dose of 10 mg/kg b.w., the serum concentration of cefquinome exceeded the MIC for most sensitive pathogens for longer time. The persistence of antibiotic concentrations in serum and tissues above the MIC is the pharmacodynamic variable related to the clinical efficacy of cefquinome.\(^{[13]}\)

In this study following intramuscular and oral administration, the peak plasma concentration (\(C_{\text{max}}\)) were 9.05 and 8.12 µg/ml, respectively, these results were reasonably similar to that reported in ducks and rabbits,\(^{[24,26]}\) but higher than that reported in chickens.\(^{[21]}\)

The values of area under the serum concentration-versus-time curve for cefquinome were 43.26 and 34.69 µg.h/ml following i.m. and oral administration, respectively, the reported values were inconsistent with that reported for chickens 5.13 µg.h/ml, ducks 23.78 µg.h/ml following i.m administration.\(^{[21,24]}\)

The elimination half-life of cefquinome after intramuscular administration to rabbits in this study (mean, 2.6 hours) was significantly longer than the value of that variable after oral administration of that drug (mean, 2.32 hours). This difference in values was likely attributable to continued absorption of cefquinome from the intramuscular injection site during the elimination phase, which would have increased the elimination half-life of the drug. The reported value of the elimination half-life following intramuscular injection was
lower than reported in piglets and camels 4.36 and 10.24 hr, respectively, \cite{34,35} and higher than that reported in ducks, chickens and sheep 1.79, 1.35 and 1.88 h, respectively. \cite{21,24,36}

Following intramuscular and oral administration of cefquinome, $t_{\text{1/2ab}}$ were 0.28 and 0.26 hr, respectively, suggesting the absorption of cefquinome following both route of administration was rapid. These results were lower than that of the plasma disposition in piglets deduced from the value of $t_{\text{1/2ab}}$ 0.41 hr, \cite{35} indicating the absorption of cefquinome in rabbits was more rapid than that in piglets.

The minimum inhibitory concentrations (MICs) of cefquinome for bacteria isolated from rabbits have not yet been figured out. The $\leq 0.1 \mu g /mL$ of cefquinome showed senior antibacterial activities against most pathogenic bacteria from calves, cattle, and pigs, which caused sepsis, pneumonia, colibacillosis, and meningitis.\cite{34} Typically, for cephalosporin antimicrobials, the time during which the circulating drug concentration remains above the MIC of an organism is the pharmacokinetic-pharmacodynamic parameter that is most highly correlated with clinical efficacy of the drug.\cite{13} However, pharmacokinetic-pharmacodynamic indices predictive of clinical efficacy that are determined via in vivo testing are determined on the basis of unbound serum drug concentrations and not on the basis of total serum or tissue drug concentrations, which are important variables for determination of the efficacy of antimicrobial drugs such as cefquinome. Such pharmacokinetic-pharmacodynamic markers should be used with caution for determination of efficacy of cephalosporins because tissue pharmacokinetic properties of such drugs are important for prediction of clinical efficacy. In the present study, the time above $\leq 0.1 \mu g /mL$ of serum concentration was approximately 12 and 10 hr following intramuscular and oral administration, respectively. Cephalosporins showed bacteriostatic activity at serum levels above the MIC for 30–40% of the dosing interval. Whereas approached bactericidal activity at the levels above the MIC for 60–70% of the time.\cite{15} In animals infected with Streptococcus pneumoniae, mortality was close to 100% if T $> \text{MIC}$ was $\leq 20\%$ of the dosage interval, but 90–100% survival was reached when T $> \text{MIC}$ was $\geq 40–50\%$ of this interval.\cite{13} Therefore, these pharmacokinetics determination suggest that cefquinome against susceptible bacterial pathogens with a MIC of $\leq 0.1 \mu g /mL$ might be recommend twice a day dose authorities at a dosage of 10 mg / kg via an intramuscular or oral route to rabbits to achieve clinically effective serum concentrations of the drug. However, further studies are needed to clear up \textit{in vitro} and \textit{in vivo} germ-killing effects and toxicological properties of cefquinome before its application in rabbits.
CONCLUSION
The pharmacokinetic results revealed that cefquinome administered to rabbits by intramuscular and oral route demonstrated good absorption and long elimination half-life ($t_{1/2}$). These pharmacokinetic attributes are highly appropriate for an antimicrobial drug indicated for the treatment of bacterial and Mycoplasma respiratory diseases in rabbits. As a result of the previous pharmacokinetic characters it's obvious that a full course therapy can be obtained after twice a day dose authorities at a dosage of 10 mg/kg of cefquinome. This is advantageous for ensuring a successful treatment outcome, when used for prevention or treatment systemic diseases in rabbits, where it is desirable to minimize stress induced by repeated handling, particularly when animals are debilitated by respiratory disease.

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REFERENCES


