Pharmacokinetic profile and some pharmacodynamic aspects of cefquinome in chickens

A. A. M. El-Gendy*, M. A. Tohamy, Abeer M. Radi

Department of Pharmacology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.

The pharmacokinetic profile and some pharmacodynamic aspects of cefquinome were studied after intramuscular (IM) and subcutaneous (SC) administration of a single dose of 2 mg kg⁻¹ b.w.t. in chickens. Tissue distribution and residues of cefquinome after repeated IM injection for 5 consecutive days were also estimated. Cefquinome was rapidly absorbed after IM and SC injection as indicated by short half-lives of absorption (t_{0.5ab}) of 0.170 and 0.262 h, respectively, while the elimination half-lives (t_{0.5el}) were 3.428 and 25.023 h, respectively. Repeated IM doses of cefquinome (2 mg kg⁻¹ b.w.t., once daily) for 5 consecutive days caused no change in serum enzyme activities of ALT and AST, but induced significant increase in serum uric acid concentration after 72 to 120 hours of administration. The withdrawal time of cefquinome from tissue of chickens is 5 days following the last dose. Cefquinome has a wide spectrum of activity against Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa.

The cephalosporins are well-known and very useful classes of antibacterials, widely used in veterinary medicine for preventing and treating bacterial infections (Becker et al., 2004). They are described as β-lactam antibiotics, based on their common chemical structure, containing the β-lactam ring. A major advantage of the β-lactam antibiotics is their high degree of safety in the target animal (Preston, 1992). Cefquinome, an aminothiazolyl cephalosporin and a member of the 4th generation of cephalosporins which have been developed especially for use in animals, has a very broad spectrum of activity against many bacteria (Guerin-Faublée et al., 2003). The in vitro and in vivo efficacy of this drug against a wide range of gram-negative and gram-positive bacteria has been demonstrated by Limbert et al., (1991). In comparison with the third generation cephalosporins, cefquinome showed a higher activity against Gram-negative bacteria and a lower affinity for plasmid-mediated cephalosporinases (Suhren and Knappstein, 2003 and Rose et al., 2004).

The aim of the present work is under taken to study the pharmacokinetics of cefquinome after single intramuscular and subcutaneous dosage in chickens. Studying the tissue residues of the drug after the repeated IM doses and its effect on liver and kidney functions and the effect on some field bacterial isolates affecting chickens were also investigated.

Material and methods

Drugs. Cefquinome was obtained from Intervet International Company, Cairo, Egypt as 2.5 cefquinome suspension in ethyl oleate (Cobactan® 2.5 %).

Chickens. Twenty four birds of both sexes with an average body weight from 1.280-2.800 kg and from 4-12 months old were used for pharmacokinetic studies and twenty four one-day old Fayoumy chicks were used for pharmacodynamic studies. These birds were obtained from El-Azab project for poultry production in Fayoum Governorate. The chickens were fed on balanced commercial ration and water ad-libitum. They were kept under good hygienic conditions and left for 15 days before the experiment for acclimatization and ensuring complete clearance of any antibacterial drugs.

Experimental protocol. Single dose pharmacokinetic studies were done on twenty four chickens which classified into two groups (each of 12 chickens). The 1st group was administered cefquinome in a single dose of 2 mg kg⁻¹ b.wt. (Block, 1996) by intramuscular route while, the 2nd group administered cefquinome in a single dose of 2 mg kg⁻¹ b.wt. by subcutaneous route. Blood samples (1ml each) were withdrawn from the wing vein just before and 0.083, 0.167, 0.250, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post drug administration. Blood samples were left to clot then centrifuged at 3000 rpm for 15 minutes to obtain clear serum that was kept frozen at -20 °C until assayed.

* Corresponding author. Tel.: +20 0822322066; fax: +20 822327982; E-mail address elgendy@bsu.edu.eg (Abdel-Nasser El Gendy).
Repeated dose pharmacokinetics were performed on 24 chickens given 2 mg kg\(^{-1}\) b.wt cefquinome intramuscularly once daily for five successive days. The blood samples were collected just before and 1 hour after dose (peak and trough). Three chicken were slaughtered at 4, 8, 12, 24 hours and 7\(^{th}\), 8\(^{th}\), 9\(^{th}\), 10\(^{th}\) days after the last dose.

Blood and tissue samples (lung, spleen, liver, kidney, breast, thigh muscle and intestine) were taken from the slaughtered chicken. One gram was taken from each tissue sample, then was thoroughly homogenized in 4 ml distilled water. Then homogenized tissue was centrifuged at 3000 revolution per minute for 15 minutes. The supernatant was transferred to sterilized tubes to be used in the assay of concentration. The serum collected from blood samples were divided into two portion, the first to be used in the assay of concentration and the second for biochemical studies. The effect of the drug on the activities of ALT and AST and concentration of uric acid were estimated according to Reitman and Frankle (1957) and Kageyama, (1971), respectively.

Bacteriological samples were taken from 50 one-day old chicks for isolation of pathogenic bacteria according to Collee et al., (1996). The microorganisms isolated from the chicks were examined for antimicrobial sensitivity against cefquinome using the disc and agar diffusion method as described by Collee et al., (1996). All the suspected microorganisms were subjected to serotyping by slide agglutination test using standard polyvalent and monovalent *E. coli* antiserum and according to the method described by Edwards and Ewing (1972). The minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) were estimated according to Collee et al., (1996).

**Drug bioassay.** Samples were assayed by microbiological assay according to the method of Arret et al., (1971) using *Micrococcus luteus* (ATCC 9341) as a test organism (San Martin et al., 1998). Standard cefquinome concentrations of 0.625, 1.25, 2.5, 5, 10, 20 and 40 \(\mu\)g ml\(^{-1}\) were prepared in antibiotic-free chicken's serum and also in distilled water. Semi-logarithmic plots of the inhibition zone diameter versus standard cefquinome concentrations in serum and distilled water were linear with typical correlation coefficient of 0.989 (for the standard curve). The difference of inhibition zone diameter between the solutions of the drug in serum and distilled water was used to calculate the *in-vitro* protein binding tendency of both drugs according to Lorian, (1980) by the following equation:

\[
\text{Protein binding %} = \frac{\text{Zone of inhibition in buffer} - \text{Zone of inhibition in serum} \times 100}{\text{Zone of inhibition in buffer}}
\]

**Pharmacokinetic analysis.** Serum concentration (log\(_{10}\)) versus time curves were generated and best fitted by the aid of computer polynomial curve stripping program (R- strip, Micromath, Scientific software, USA). Data from each animal were fitted individually and the pharmacokinetic variables were computed by the aid of the software program. The first order absorption and elimination rate constants (\(K_{ab}\) and \(K_{el}\)) and the corresponding extrapolated zero time intercepts (A and B), elimination and absorption half lives (\(t_{0.5(el)}\) and \(t_{0.5(ab)}\)), mean residence time (MRT), maximum serum concentration (C\(_{max}\)) and time to be achieved (t\(_{max}\)) were also estimated. The area under the serum concentration-time curve (AUC) was calculated by trapezoidal rule. Results were expressed as mean and standard error (S.E). Standard errors were calculated from the mean data according to Snedecor, (1969).

**Results**

The diagrammatic relation between the time and the observed concentrations of cefquinome after IM and SC administration of 2 mg kg\(^{-1}\) b.wt were demonstrated in figure (1). The pharmacokinetic parameters of cefquinome after IM and SC routes are presented in table (1).

Following intramuscular and subcutaneous injections, cefquinome was rapidly absorbed with a half-lives of absorption (\(t_{0.5(ab)}\)) of 0.170 and 0.262 h and the peak serum concentrations (C\(_{max}\)) were 12.421 and 4.935 \(\mu\)g ml\(^{-1}\), respectively. The elimination half-lives (\(t_{0.5(el)}\)) were 3.428 and 25.023 h., respectively. *In-vitro* protein binding percent in chicken's serum ranged from 2.89-18.27 (mean 6.67) %.

Serum concentrations of cefquinome following multiple intramuscular administration of 2 mg kg\(^{-1}\) b.wt. in chickens for 5 consecutive days were illustrated in figure (2). Multiple dose studies have demonstrated that cefquinome was cumulative over 5 days with a 24 hour dosing regimen. Table (2) demonstrate the serum and tissue concentration of the drug after multiple dosing. Cefquinome was not detected in any tissues except kidney after 120 hours following the last dose. Repeated IM administration of cefquinome (2 mg kg\(^{-1}\) b.wt once daily) for 5 consecutive days caused no change in serum enzyme activities of ALT and AST, but induced a significant increase in concentration of serum
Table (1): Mean (± SE) kinetic parameters of cefquinome (2 mg kg\(^{-1}\) b.wt) following a single IM and SC administration in chickens (n=12).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>IM</th>
<th>S.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>k(_{ab})</td>
<td>h(^{-1})</td>
<td>4.248 ± 0.256</td>
<td>3.290 ± 0.426</td>
</tr>
<tr>
<td>K(_{el})</td>
<td>h</td>
<td>0.226 ± 0.020</td>
<td>0.035 ± 0.005</td>
</tr>
<tr>
<td>t(_{0.5(ab)})</td>
<td>h</td>
<td>0.170 ± 0.011</td>
<td>0.262 ± 0.042</td>
</tr>
<tr>
<td>t(_{0.5(el)})</td>
<td>h</td>
<td>3.428 ± 0.374</td>
<td>25.023 ± 3.28</td>
</tr>
<tr>
<td>C(_{max})</td>
<td>ug ml(^{-1})</td>
<td>12.421 ± 0.753</td>
<td>4.935 ± 0.270</td>
</tr>
<tr>
<td>t(_{max})</td>
<td>h</td>
<td>0.227 ± 0.007</td>
<td>0.292 ± 0.022</td>
</tr>
<tr>
<td>AUC</td>
<td>ug ml(^{-1}) h(^{-1})</td>
<td>17.585 ± 0.815</td>
<td>5.599 ± 0.360</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>3.636 ± 0.421</td>
<td>20.680 ± 4.615</td>
</tr>
</tbody>
</table>

k\(_{ab}\), first-order absorption rate constant; K\(_{el}\), elimination rate constant; C\(_{max}\), maximum serum concentration; t\(_{max}\), time to peak serum concentration; t\(_{0.5(ab)}\), absorption half-life; t\(_{0.5(el)}\), elimination half-life; MRT, mean residence time; AUC\(_{0-12}\), area under serum concentration-time curve.

Table (2): Mean serum and tissue concentrations (ug ml\(^{-1}\)) of cefquinome (2 mg kg\(^{-1}\) b.wt twice daily) in chickens after the last dose of repeated IM doses (n=3).

<table>
<thead>
<tr>
<th>Time of slaughter</th>
<th>4 h</th>
<th>8 h</th>
<th>12 h</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>2.09±0.69</td>
<td>1.31±0.20</td>
<td>1.02±0.17</td>
<td>1.69±0.29</td>
<td>0.352±0.35</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>7.312±0.43</td>
<td>5.631±0.57</td>
<td>3.616±0.25</td>
<td>2.82±0.032</td>
<td>2.098±0.22</td>
<td>ND</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.167±0.72</td>
<td>4.67±0.13</td>
<td>3.709±0.10</td>
<td>2.226±0.87</td>
<td>1.908±0.66</td>
<td>0.149±0.15</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.708±0.34</td>
<td>2.73±0.21</td>
<td>2.18±0.13</td>
<td>1.385±0.31</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lung</td>
<td>5.826±1.96</td>
<td>4.869±0.77</td>
<td>3.709±0.59</td>
<td>2.488±0.34</td>
<td>0.258±0.26</td>
<td>ND</td>
</tr>
<tr>
<td>Intestine</td>
<td>5.331±1.083</td>
<td>4.083±1.16</td>
<td>3.965±0.47</td>
<td>1.974±1.09</td>
<td>2.018±0.42</td>
<td>ND</td>
</tr>
<tr>
<td>Breast muscle</td>
<td>5.572±1.52</td>
<td>4.825±1.13</td>
<td>3.386±0.31</td>
<td>2.103±0.50</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Thigh muscle</td>
<td>ND</td>
<td>ND</td>
<td>2.258±0.81</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not detected

Figure (1): Semilogarithmic graph depicting the time-concentration of cefquinome in serum of chickens after a single IM and SC injection of 2 mg/kg b.wt.

Figure (2): Semilogarithmic plot depicting the time-course of cefquinome in serum of chickens after repeated intramuscular injection of 2 mg kg\(^{-1}\) b.wt. once daily for 5 consecutive days.
uric acid at 72 to 120 hours of administration.

From the bacteriological study, the microorganisms recovered from the chicks were *Escherichia coli* O78 serogroup, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Cefquinome at concentration of (10 ug/well) inhibited the growth of all examined microorganisms. The minimum concentrations of cefquinome which inhibited the growth of *Escherichia coli* O78, *Proteus mirabilis* and *Pseudomonas aeruginosa* were 0.5, 1 and 16 ug/ml. The minimum bactericidal concentrations (MBC) of cefquinome which killed the tested microorganisms were 1.16 and >128 ug/ml.

**Discussion**

Following intramuscular injection of cefquinome in a single dose of 2 mg kg⁻¹ b.wt, the drug was rapidly absorbed with an absorption half-life (t₀.₅(ab)) 0.170 h and slowly eliminated with an elimination half-life (t₀.₅(el)) 3.428 h. These finding were similar to those reported by Maha, (2005), 0.153 and 4.84 h, respectively. The study recorded long elimination half-life (t₀.₅(el)) of cefquinome after subcutaneous injection 25.023 h. After repeated intramuscular injection of a dose 2 mg kg⁻¹ b.wt once daily for 5 consecutive days, the results indicated that cefquinome was accumulated in the body. The drug was detected in most examined tissues up to 72 h after the last dose. It has been shown that cefquinome was poorly bound to plasma protein (6.665%) which is similar to that reported by Limbert et al., (1991) in mouse, dog, horse and calf which less than 10 %. The rapid absorption and lower protein binding of cefquinome after intramuscular injection gave the ability to induce rapid effect by this route and may explain high diffusion of the drug in tissues of chickens.

In this study, the drug concentrations of cefquinome in serum exceeded the MIC of *E Coli* and *Proteus mirabilis*, but less than the MIC of *pseudomonas aeruginosa*. Cruickshank et al., (1975) considered that a bacterium may be sensitive to antibiotic if the MIC is not more than 0.25-0.5 its average concentration in blood.

It could be concluded that cefquinome has advantageous pharmacokinetic profile following its I/M administration to chickens at 2 mg kg⁻¹ b.wt. Moreover, it has a wide spectrum of activity against *Escherichia coli* O78, *Proteus mirabilis* and *Pseudomonas aeruginosa*. In addition, it does not produce hepatic toxicity, but causes mild renal toxicity.

**References**


المسار الحركي وبعض الجوانب الفارماكوديناميكية لعقار سيفينيتيوم في الدواجن

تم دراسة المسار الحركي لسيفينيتيوم (2 مجم/كجم من وزن الجسم) في الدجاج. كما تم تعيين معدل الاستفادة الحيوية من العقار وتعيين نسبة اتخاذ بروتينات العضل وتكراره بعد الحقن المكرر في المصل والأنسجة ودراسة تأثيره على نشاط بعض إنزيمات العضل وتركيز حمض الديكسيك. وتم دراسة تفاعل وتأثير العقار على بعض الميكروبات داخل العضل. وقد أظهرت الدراسة أنه بعد الحقن العضلي تحت الجلد كان أقل تركيز للدواء (Cmin، 12.421 و4,935 ميكروجرام/مليليو folks) عند زمن 0.170 و0.227 (t1/2) ساعة من الحقن على التوالي. وقد كانت فترة نصف عمر الانصمام (t1/2) 25.32 و2.238 ساعة على التوالي. ووجد أن الدواء لا يحدث تغير في نشاط بعض إنزيمات العضل (ALT and AST). كما وجد أنه قد ينخفض من العقار من كل كبسولة الجسم بعد 120 ساعة من الحقن. من الاختبار المعمل أنتج أن ميكروبات البروتوفيلير ميراباس والقولونين الأنثروبوري (الأميريكي) والزائفة الزنجارية أكثر حساسية لسيفينيتيوم وأن أقل تركيز من العقار والذي ينخفض نمو هذه البكتيريا هو 0.5 و1 و16 و128 والتي يتطلب هذه البكتيريا 0.5 و1 و16 و128 لاستخدامها.