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Pharmacokinetic profile and some pharmacodynamic aspects of cefquinome in chickens

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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.wt. 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 ($t_{0.5(ab)}$) of 0.170 and 0.262 h., respectively, while the elimination half-lives ($t_{0.5(el)}$) were 3.428 and 25.023 h., respectively. Repeated IM doses 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 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 *Esherchia coli, Proteus mirabilis* and *Pseudomonas aeroginosa*.

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-Faublee *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.

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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 day 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 0.083,0.167,0.25,0.5,1,2,4,6,8,12 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.

Repeated dose pharmacokinetics were performed on 24 chickens given 2 mg kg^{-1} b.wt cefquinome intramuscularlly once daily for five successive days. The blood samples were collected just before and 1 hour after dose (peak and trough). Three chicken were slaughterd 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 at 3000 revolution per minute for 15 minutes. The supernatent 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 diffussion 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* antisera 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 ug 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:

Protein binding % =[Zone of inhibition in buffer – Zone of inhibition in serum] X 100/ Zone of inhibition in buffer

Pharmacokinetic analysis. Serum concentration (log_{10}) versus time curves were generated and best fitted by the aid of computer poly-exponential 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 ug 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 regimens. 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).

Parameter	Unit	I.M	S.C
k_{ab}	h^{-1}	4.248 ± 0.256	3.290 ± 0.426
K_{el}	h^{-1}	0.226 ± 0.020	0.035 ± 0.005
$t_{0.5(ab)}$	h	0.170 ± 0.011	0.262 ± 0.042
$t_{0.5(el)}$	h	3.428 ± 0.374	25.023 ± 3.28
C_{max}	ug ml^{-1}	12.421 ± 0.753	4.935 ± 0.270
t_{max}	h	0.227 ± 0.007	0.292 ± 0.022
AUC	ug ml^{-1} h^{-1}	17.585 ± 0.815	5.599 ± 0.306
MRT	h	3.636 ± 0.421	20.680 ± 4.615

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).

	Time of slaughter					
	4 h	8 h	12 h	24 h	72 h	120 h
Serum	2.09±0.69	1.31±0.20	1.02±0.17	1.69±0.29	0.352±0.35	ND
Liver	7.312±0.43	5.631±0.57	3.616±0.25	2.82±0.032	2.098±0.22	ND
Kidney	5.167±0.72	4.67±0.13	3.709±0.10	2.226±0.87	1.908±0.66	0.149±0.15
Spleen	3.708±0.34	2.73±0.21	2.184±0.13	1.385±0.31	ND	ND
Lung	5.826±1.96	4.869±0.77	3.709±0.59	2.488±0.34	0.258±0.26	ND
Intestine	5.331±1.083	4.083±1.16	3.965±0.47	1.974±1.09	2.018±0.42	ND
Breast muscle	5.572±1.52	4.825±1.13	3.386±0.31	2.103±0.50	ND	ND
Thigh muscle	ND	ND	2.258±0.81	ND	ND	ND

ND = Not detected

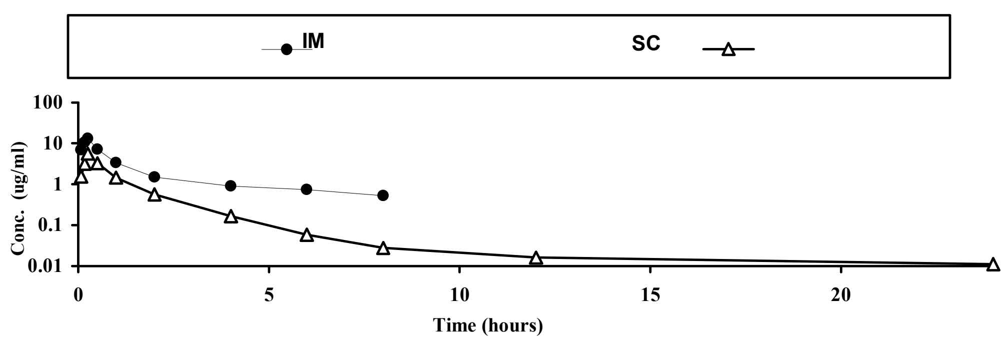

Figure (1): Semilogarithmic graph depicting the time-concentration of cefquinome in serum of chickens after a single im and sc injection of 2 mg/kg b.wt

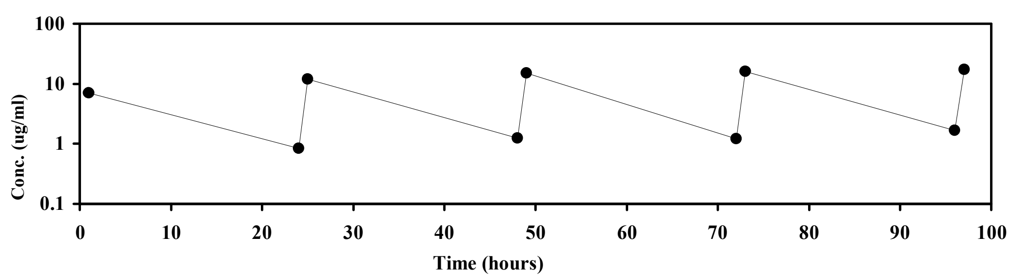

Figure (2) : Semilogarithmic plot depicting the time-course of cefquinome in serum of chickens after repeated intramuscular injection of 2 mg kg^{-1} b.wt. once daily for 5 consecutive days

uric acid at 72 to 120 hours of administration.

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

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 ($t_{0.5(ab)}$) 0.170 h and slowly eliminated with an elimination half-life ($t_{0.5(el)}$) 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 ($t_{0.5(el)}$) 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 aeroginosa*. Cruichshank *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 *Esherchia coli* O78*, Proteus mirabilis* and *Pseudomonas aeroginosa*. In addition, it does not produce hepatic toxicity, but causes mild renal toxicity.

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المسار الحركي وبعض الجوانب الفارماكوديناميكية لعقار سيفكينوم في الدواجن

تم دراسة المسار الحركي للسيفكينوم (2 مجم/كجم من وزن الجسم) في الدجاج. كما تم تعيين معدل الاستفادة الحيوية من العقار وتعيين نسبة إتحاده ببروتينات المصل وتركيزه بعد الحقن المتكرر في المصل والأنسجة ودراسة تأثيره على نشاط بعض إنزيمات المصل وتركيز حمض البوليك. وتم دراسة نشاط وتأثير العقار عل بعض الميكروبات داخل المعمل. وقد أظهرت الدراسة انه بعد الحقن العضلي و تحت الجلد كان أقصى تركيز للدواء (C_{max}) 12.421 و 4.935 ميكروجرام / مللي على التوالي وبعد زمن (t_{max}) 0.227 و 0.292 ساعة من الحقن على التوالي. وقد كانت فترة نصف عمر الامتصاص ($t_{0.5(ab)}$) 0.170 و 0.262 ساعة وفترة نصف عمر الإخراج ($t_{0.5(el)}$) 3.428 و 25.023 ساعة على التوالي. ووجد أن الدواء لا يحدث تغيير في نشاط بعض إنزيمات المصل (ALT and AST). كما وجد أنة قد تم التخلص من العقار من كل أنسجة الجسم بعد 120 ساعة من الحقن. من الاختبار المعملي أتضح أن ميكروبات البروتيس ميرابيلس والقولون الأشريكى (الأشيرشياكولى) والزائفة الزنجارية أكثر حساسية للسيفكينوم وأن اقل تركيز من العقار والذي يوقف نمو هذه البكتريا هو 0.5 و1 و16 واقل تركيز يقتل هذه البكترياهو1 و16 و>128 على التوالي.