Vol. 121: 29–35, 2016
doi: 10.3354/dao03032

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published August 31

Ceftiofur pharmacokinetics in Nile tilapia *Oreochromis niloticus* after intracardiac and intramuscular administrations

Waleed F. Khalil[1,*], Hazem M. Shaheen[2], Rania H. Abdou[3]

[1]Pharmacology Department, Faculty of Veterinary Medicine, University of Suez Canal, Ismailia 41522, Egypt
[2]Pharmacology Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt
[3]Departments of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, University of Suez Canal, Ismailia 41522, Egypt

ABSTRACT: Ceftiofur is a broad-spectrum third generation cephalosporin, which acts by inhibiting bacterial cell wall synthesis. It is active against Gram-positive and Gram-negative bacteria such as *Aeromonas hydrophila* and β-lactamase-producing strains, which are common pathogens in freshwater fish. Ceftiofur pharmacokinetics in Nile tilapia *Oreochromis niloticus* were studied following single intracardiac (i.c.) or intramuscular (i.m.) administration of ceftiofur sodium (NAXCEL®) in a dose of 5 mg ceftiofur kg^{-1} body weight. After i.c. injection, ceftiofur plasma concentrations decreased biexponentially, suggesting a 2-compartmental open model. Distribution and elimination half-lives ($t_{0.5(\alpha)}$ and $t_{0.5(\beta)}$) were 0.61 ± 0.22 and 0.14 ± 0.03 h mean ±SD, respectively. Elimination constant (K_{el}) and total body clearances (Cl_{tot}) were 3.22 ± 0.48 h^{-1} and 1.64 ± 0.47 l h^{-1} kg^{-1}, respectively. Volume of distribution (Vss) and areas under curves (AUC) were 0.12 ± 0.03 l kg^{-1} and 24.18 ± 8.81 µg ml^{-1} h, respectively. Following i.m. injection of ceftiofur, plasma concentrations were best described by a 1-compartment open model with a first order absorption; bioavailability was quite high (96.85 ± 23.74%). Plasma maximum concentration (C_{max}) was 12.32 ± 6.53 µg ml^{-1}; achieved at time of maximum concentration (T_{max}) of 0.74 ± 0.04 h. Absorption and elimination half-lives ($t_{0.5(ab)}$ and $t_{0.5(\beta)}$) were 0.49 ± 0.06 and 0.53 ± 0.03 h, respectively. In conclusion, i.m. injection of ceftiofur sodium produced extremely high bioavailability with high plasma concentrations that persisted up to 6 h post injection, which may make ceftiofur a useful alternative antibiotic for treatment of brood stock or important ornamental fishes.

KEY WORDS: Ceftiofur · Cephalosporin · Pharmacokinetics · Teleosts · Tilapia · Intracardiac · Intramuscular

INTRODUCTION

Ceftiofur is a broad-spectrum third generation cephalosporin acting by inhibition of bacterial cell wall synthesis. It is active against Gram-positive and Gram-negative pathogens of veterinary importance, including β-lactamase-producing strains (Yancey et al. 1987) and *Aeromonas hydrophila* (Dixon & Issvoran 1992).

The pharmacokinetics of ceftiofur has been studied in many animal species including cattle (Brown et al. 1996, Foster et al. 2016), horses (Jaglan et al. 1994, Fultz et al. 2015), sheep (Rivera-Garcia et al. 2014), dairy goats (Garrett et al. 2015), camels (Goudah 2007), swine (Day et al. 2015), dogs (Brown et al. 1995a), and finally, mice (Brown et al. 1995b).

To our knowledge, however, there is lack of data about the ceftiofur kinetics of fish (Grosset et al.

*Corresponding author: wk@vet.suez.edu.eg

2015), although other cephalosporins, such as cefquinome and cephalexin, have been the objects of a detailed pharmacokinetic study in salmon and sea bream, respectively (San Martin et al. 1998, Katharios et al. 2004).

Nevertheless, ceftiofur is currently used to control a wide range of bacterial infections in ornamental fish. Ceftiofur sodium was found to be highly effective against 42 isolates of *Aeromonas* species isolated from ornamental fishes (Dixon & Issvoran 1992).

In freshwater fish farms, diseases caused by *A. hydrophila* are spread worldwide and considered to be a major economic problem (Austin & Austin 2012). In aquaculture, medications are mainly used for controlling bacterial diseases in tilapia cultures, which may result in environmental pollution with antibiotics through leaching of antibiotics from uneaten fish feed or through tilapia excrements (Robinson et al. 2007). This may result in adverse ecological effects, including the development of resistant bacterial populations, direct toxicity to microflora and microfauna, and/or possible risks in the transfer of antibiotic resistance to human pathogenic microbes (Hektoen et al. 1995, Rigos et al. 2004, Cabello 2006).

The objective of this paper was to obtain information on the pharmacokinetic properties of ceftiofur sodium in plasma of tilapia, after intracardiac (i.c.) or intramuscular (i.m.) administration at a single dose regimen, using microbiological assay.

MATERIALS AND METHODS

Drug

Ceftiofur was administered in the form of the commercially available preparation of ceftiofur sodium (NAXCEL® sterile powder vials, Pharmacia & Upjohn Co.) after reconstitution in sterile distilled water to achieve a final concentration of 1 mg ceftiofur equivalents ml^{-1}.

Fish

A total of 80 tilapia fish *Oreochromis niloticus* was obtained from El-Salam water way (Port Said, Egypt) and kept for 2 wk for acclimatization in glass aquaria under controlled conditions with a photoperiod of 16 h light: 8 h dark. Fish body weights and total body lengths were 136.4 ± 17.2 g and 14.1 ± 1.2 cm (mean ± SD), respectively. After acclimatization, tilapia were subdivided into 2 groups; one group was used for i.c. and the other for i.m. drug injection. Each group contained 5 replicates (8 fish per replicate); every replicate was kept in a separate water tank (pH 6.8–7, 22–25°C and 5.2–5.9 mg l^{-1} dissolved oxygen). Tap water was used after dechlorination, filtration, and salinity adjustment. Fish were fed an antibiotic-free diet at a ratio of 3% of their body weight d^{-1}; feed (37–40% protein) was obtained from the Faculty of Agriculture, Suez Canal University, Egypt. Feed was withheld for 24 h before drug administration.

Drug administration, and sample collection

Fish (randomly chosen) were weighed and anesthetized in benzocaine bath (100 mg l^{-1} for 5 min exposure) after being captured. Ceftiofur sodium was administered either i.c. or i.m. (epaxial muscle ~1 cm below the dorsal fin) in a single dose of 5 mg ceftiofur equivalents kg^{-1} body weight using a 24 gauge needle.

For blood collection, 5 fish were removed from the treated tank at each sampling time and anesthetized, ~0.5 ml of blood was extracted from the caudal vein, using a heparinized syringe at 0.08, 0.25, 0.5, 0.75, 1, 2, 3, and 4 h after i.c. and at 0.17, 0.5, 1, 2, 3, 4, 5, and 6 h after i.m. drug administration. All blood samples were centrifuged at 2600 × *g* for 10 min, and plasma was collected and stored at −20°C until assaying.

Quantitative assay of Ceftiofur

Ceftiofur concentrations were assayed microbiologically by well diffusion methods using nutrient meat-peptone agar medium and *E. coli* ATCC 25922 as a test microorganism. Ceftiofur standard solutions were prepared in plasma from untreated fish as a reference. The detection limit of the assay was 0.07 µg ml^{-1}. The standard curve showed a linear relationship over the range of 0.07 to 6 µg ceftiofur ml^{-1} with a correlation coefficient (r) of 0.981. Intra- and inter-assay coefficient of precision were 3.6 and 5.2%, respectively.

Pharmacokinetics and statistical analysis

Compartmental analysis of ceftiofur in plasma was performed using a nonlinear regression analysis program WinNonlin (Version1.1, Pharsight). Classical pharmacokinetic parameters were calculated using standard equations (Gibaldi & Perrier 1982). The bioavailability (*F*, in percent) was calculated using the equation:

$$F = (AUC_{i.m.}/AUC_{i.c.}) \times 100$$

where AUC is the area under curves. Predicted plasma concentration (conc.) at a given time after i.c. ceftiofur injection was calculated based on the following 2-compartmental pharmacokinetic equation:

$$\text{Conc.} = A \times \exp(-\alpha \times T) + B \times \exp(-\beta \times T) \quad (1)$$

where T is time in hours, A and B are zero-time plasma concentration intercepts of the biphasic disposition curve, and α and β are distribution and elimination rate constants, respectively. Predicted concentration (conc.) at a given time after i.m. injection were calculated according to the following 1-compartment (with a first order absorption) pharmacokinetic equation:

$$\text{Conc.} = D \times K_{01} \times V^{-1} \times (K_{01} - K_{10}) \times [\exp(-K_{10} \times T) - \exp(-K_{01} \times T)] \quad (2)$$

where D and V are administered dose and volume of distribution, respectively, K_{01} and K_{10} are absorption and elimination rate constants, respectively, and T means time in hours. The arithmetic mean and standard deviation were calculated for all parameters except for half-life values where harmonic mean values and standard deviation were calculated according to Lam et al. (1985).

RESULTS

All fishes were clinically healthy throughout the experiment. There were no identifiable reactions following the administration of ceftiofur. Ceftiofur plasma concentration–time profiles following single i.c. or i.m. administration in a dose of 5 mg ceftiofur kg^{-1} body weight in Nile tilapia are shown in Figs. 1 & 2, respectively.

After i.c. injection, ceftiofur plasma concentrations decreased biexponentially, suggesting the 2-compartmental open model, while ceftiofur plasma concentrations after i.m. injection were best described by a 1-compartment open model with a first order absorption. The pharmacokinetic parameters describing ceftiofur disposition after i.c. or i.m. administration are listed in Tables 1 & 2, respectively.

Following i.c. administration of ceftiofur sodium, rapid distribution and penetration patterns were achieved (i.e. the volume of distribution at steady state [Vdss], the distribution and penetration rates (k_{12}, k_{21}), and the tissue-to-plasma-level ratio at the peak tissue level (k_{12}/k_{21}). These findings significantly ($p \leq 0.05$) prolonged the distribution ($t_{0.5(\alpha)}$) of

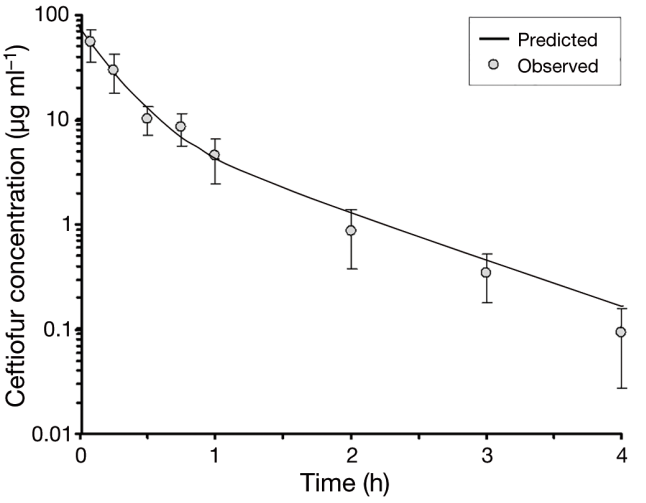

Fig. 1. Semilogarithmic plot of predicted (solid line) and observed (gray circles) ceftiofur plasma concentrations versus time after single intracardiac administration of 5 mg ceftiofur kg^{-1} body weight in *Oreochromis niloticus*. Observed values represent means ± SD (n = 5) at each time point. Predicted concentrations are calculated based on Eq. (1)

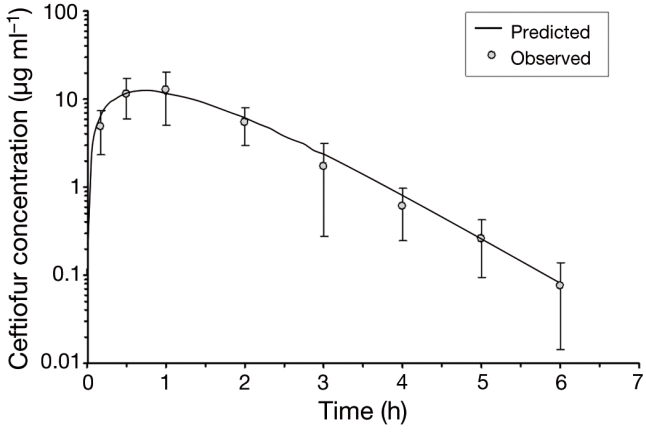

Fig. 2. Semilogarithmic plot of predicted (solid line) and observed (gray circles) ceftiofur plasma concentrations versus time after single intramuscular administration of 5 mg ceftiofur kg^{-1} body weight in *Oreochromis niloticus*. Observed values represent means ± SD (n = 5) at each time point. Predicted concentrations are calculated based on Eq. (2)

ceftiofur after intramuscular treatment (0.49 ± 0.06 h vs. 0.14 ± 0.03 h in i.c.-treated fishes).

The maximum concentrations (C_{max}) of 12.32 ± 6.53 µg ml^{-1} in plasma were reached after 0.74 ± 0.04 h (time of maximum concentration, t_{max}). After 4–6 h following ceftiofur injection by both routes, the ceftiofur plasma concentrations were below the detection limit (around 0.10 µg ml^{-1}).

Intracardiac administration with ceftiofur significantly ($p \leq 0.05$) increased the elimination half-life ($t_{0.5(\beta)}$). The $t_{0.5(\beta)}$ was 0.61 ± 0.22 h as opposed to 0.53

± 0.03 h in the i.m.-treated ones. The elimination rate constant (K_{el}) differed significantly in the i.c.-injected fishes (3.22 ± 0.48 h) compared to 1.31 ± 0.07 h in i.m.-treated group. The AUC value in i.c.-treated fish (24.18 ± 8.81 µg ml^{-1} h) was similar to that of i.m.-injected fish (24.75 ± 13.30 µg ml^{-1} h).

DISCUSSION

Fish used in the experiment tolerated the high dose well. The plasma concentration–time curve of ceftiofur in tilapia after both i.c. and i.m. administration was best described by the 2-compartment and 1-compartment open models, respectively. The kinetics of ceftiofur sodium was described by 1- or 2-compartment models in avian species (Tell et al. 1998). Absorption, distribution and elimination of the drug were fast, as already described in literature for other animal species (Carli et al. 1983, 1999, Soback et al. 1987, Garg et al. 1990, 1996, Villa et al. 2002).

Our results demonstrate a rapid absorption of intramuscular-administered ceftiofur in tilapia, with maximum plasma concentration occurring less than 1 h post-treatment. Most studies on β-lactam antibiotics indicate that efficacy depends on the persistence of the antibiotic serum concentration above the minimal inhibitory concentration (MIC) of the target pathogen for at least 12 h (Silley & Brewster 1988, Brown et al. 1999). The MIC of ceftiofur lies within the range of 0.25–1 µg ml^{-1} (Dixon & Issvoran 1992). In our study, the plasma concentration of ceftiofur exceeded the MIC several times for at least 3–5 h. Thereafter, the ceftiofur level in the plasma of the fish declined dramatically, below the limit of assay detection. The variation between k_{12} (1.12 ± 0.41) and k_{21} (1.83 ± 0.77) after i.c. ceftiofur injection indicated that the concentration of ceftiofur in the tissues is greater than in the plasma (Horsberg 1994).

The higher elimination rate constant of ceftiofur sodium following i.c. administration compared with the i.m. route highly suggested that the muscle may act as a reservoir of this drug (Horsberg 1994). In addition, biotransformation of ceftiofur sodium to desfuroylceftiofur (DFC) is more rapid when given i.c. due to the first-pass effect (Smith & Reynard 1995).

After i.c. and i.m. injection of ceftiofur in tilapia, the elimination half-lives ($t_{0.5(\beta)}$) were almost the same (0.61 ± 0.22 and 0.53 ± 0.03 h, respectively). These values are comparable with that recorded in grass carp (0.38 h) after i.c. injection of 8 mg ceftiofur kg^{-1} body weight (Somjetlertcharoen 2001). In contrast, the ceftiofur elimination half-lives in our study were much shorter than those recorded in koi fish (315.6 and 583.8 h, respectively) after i.c. and i.m. administration of extended release ceftiofur crystalline-free acid (CCFA) formula (Grosset et al. 2015). These differences between ceftiofur half-lives among tilapia and koi fishes can be attributed to the difference of ceftiofur pharmaceutical forms in our and their study more than to the difference in fish species. CCFA is

Table 1. Pharmacokinetic parameters of ceftiofur following single intracardiac administration of 5 mg ceftiofur kg^{-1} body weight in *Oreochromis niloticus*. *A* and *B*: zero-time plasma drug concentration intercepts of biphasic disposition curve; α and β: distribution and elimination rate constants, respectively; $t_{0.5(\alpha)}$ and $t_{0.5(\beta)}$: distribution and elimination half-lives, respectively; k_{12} and k_{21}: diffusion rate constant from the central compartment to the peripheral compartment and vice versa; Cl_{tot}: total body clearance; MRT: mean residence time of drug molecules in chicken body; Vdss: volume of distribution at steady state; AUC: area under the drug plasma concentration-time curve; AUMC: area under the moment curve

Parameter	Unit	Mean ± SD
A	µg ml^{-1}	64.82 ± 27.31
α	h^{-1}	5.04 ± 1.01
$t_{0.5(\alpha)}$	h	0.14 ± 0.03
B	µg ml^{-1}	11.47 ± 4.46
β	h^{-1}	1.13 ± 0.33
$t_{0.5(\beta)}$	h	0.61 ± 0.22
k_{12}	h^{-1}	1.12 ± 0.41
k_{21}	h^{-1}	1.83 ± 0.77
K_{el}	h^{-1}	3.22 ± 0.48
Cl_{tot}	l kg^{-1} h^{-1}	1.64 ± 0.47
MRT	h	0.52 ± 0.07
Vdss	l kg^{-1}	0.12 ± 0.03
AUC	µg ml^{-1} h	24.18 ± 8.81
AUMC	µg ml^{-1} h^2	12.93 ± 6.18

Table 2. Pharmacokinetic parameters of ceftiofur following single intramuscular administration of 5 mg ceftiofur kg^{-1} body weight in *Oreochromis niloticus*. C_{max}: maximum drug concentration; T_{max}: time at which C_{max} was achieved; $t_{0.5(ab)}$ and $t_{0.5(\beta)}$: absorption and elimination half-lives, respectively; K_{ab} and K_{el}: absorption and elimination rate constants, respectively; AUC: area under the drug plasma concentration–time curve; $F_{i.m.}$: intramuscular bioavailability

Parameter	Unit	Mean ± SD
C_{max}	µg ml^{-1}	12.32 ± 6.53
T_{max}	h	0.74 ± 0.04
$t_{0.5(ab)}$	h	0.49 ± 0.06
$t_{0.5(\beta)}$	h	0.53 ± 0.03
K_{ab}	h^{-1}	1.42 ± 0.20
K_{el}	h^{-1}	1.31 ± 0.07
AUC	µg ml^{-1} h	24.75 ± 13.30
$F_{i.m.}$	%	96.85 ± 23.74

formulated as a suspension in caprylic/capric triglyceride and cottonseed oil-based to permit gradual and sustained release of the ceftiofur in blood (Giguere et al. 2011).

Ceftiofur showed moderate volume of distribution (0.12 ± 0.03 l kg^{-1}) in tilapia after i.c. administration; similar volumes (0.09 and 0.17 l kg^{-1}) were observed in grass carp after i.c. and intraperitoneal (i.p.) injection of ceftiofur, respectively (Somjetlertcharoen 2001). Regarding the CCFA kinetics in koi, Grosset et al. (2015) registered a huge central compartment volume of about 3.6 and 5.9 l after i.c. and i.m. drug injections, respectively. The differences between our and their calculated volume of distribution are mainly attributed to the slow release of ceftiofur from its oily base in CCFA formula. Moreover, while we used the compartmental open model to calculate ceftiofur volume of distribution, the nonlinear mixed-effects model was used to calculate the CCFA volume of distribution in koi, which in turn was greatly confounded by the variations in the ceftiofur bioavailability in koi (Grosset et al. 2015).

The area under the plasma ceftiofur concentration–time curve (AUC) represents the extent of body exposure to ceftiofur after its administration. In tilapia, ceftiofur injection by both i.c. and i.m. routes resulted in AUCs of 24.18 and 24.75 µg ml^{-1} h, respectively. These values are in accordance with those calculated in grass carp (38.51 and 30.96) after i.c. and i.m. injection of 8 mg ceftiofur kg^{-1} body weight, respectively (Somjetlertcharoen 2001).

Water temperature plays a major role in the efficiency of drug metabolism in poikilothermic animals. Water temperature also contributes to regulating absorption, distribution and excretion of drugs given to fish. Temperature-related absorption and excretion of chemotherapeutic agents have been studied *in vitro* and *in vivo* (Björklund & Bylund 1990, Bowser et al. 1992, Martinsen et al. 1992, Sohlberg et al. 1994). Lower temperatures which decrease absorption rate probably contribute to the decreased bioavailability generally seen in cold water species of fish (Bowser et al. 1992). Conversely, the relatively warm water temperature in this study (22–25°C) may have contributed to the faster absorption and elimination of ceftiofur sodium in tilapia.

The usual ceftiofur sodium treatment regimen in veterinary medicine is 1.1–2.2 mg kg^{-1} once daily for up to 5 consecutive days administered by either intravenous or intramuscular route (Brown et al. 1996). However, antibiotics that inhibit cell wall synthesis, such as the cephalosporins, work only on actively growing bacteria and are, therefore, more effective if given at longer dose intervals, allowing a short period of bacterial re-growth between doses (Cunha & Gill 1995).

The pharmacokinetic parameters assessed after parenteral administration of the drug permit consideration of cephalosporin for treatment of microbial diseases in sea bream commercial aquaculture. Absence of adverse effects even at the therapeutic dose indicates a high pharmacological manageability of the drug. Moreover, known existence of di- and tripeptide transport proteins (located at the apical and basolateral membranes of the enterocytes) that also mediate the lumen to blood transport of β-lactam antibiotics across the intestinal epithelium of teleost fish (Verri et al. 1992, 2000, Thamotharan et al. 1996a,b, Maffia et al. 1997) suggests the possibility of oral administration of this class of drugs by medicated feed, as routinely applied in fish farming. However, oral administration of ceftiofur in tilapia (5 mg ceftiofur equivalents kg^{-1} body weight) did not show any detectible plasma concentrations (authors' unpubl. results). Results from research on the tissue distribution of antibacterial agents in aquaculture should be combined with the results of environmental and toxicological investigations so as allow the best choice of an antibiotic with a high bioavailability, acceptable distribution, limited toxicity, and a quick withdrawal time. Care must be taken concerning the treatment scheme, owing to rapid elimination of the drug from plasma which would make a 3–4 times daily administration of ceftiofur necessary for efficient antimicrobial treatments.

In conclusion, although ceftiofur is recognized as a long-acting antibiotic in most animal species, including cattle, goats, pigs, chickens, dogs and horses (2.5–13 h), it has a very short half-life in tilapia; such a short half-life may considerably limit its practical use in tilapia and maybe in other fish species as well. Only in brood stock or important ornamental fishes could ceftiofur (by i.m. injection) be a useful alternative antibiotic for treatment of sensitive bacterial infection.

LITERATURE CITED

Austin B, Austin D (2012) Aeromonadaceae representatives (*Aeromonas salmonicida*). In: Austin B, Austin DA (eds) Bacterial fish pathogens: disease in farmed and wild fish. Springer, New York, NY, p 147–228

Björklund H, Bylund G (1990) Temperature-related absorption and excretion of oxytetracycline in rainbow trout (*Salmo gairdneri* R.). Aquaculture 84:363–372

Bowser PR, Wooster GA, St Leger J, Babish JG (1992) Pharmacokinetics of enrofloxacin in fingerling rainbow trout (*Oncorhynchus mykiss*). J Vet Pharmacol Ther 15:62–71

Brown SA, Arnold TS, Hamlow PJ, Speedy AK and others (1995a) Plasma and urine disposition and dose proportionality of ceftiofur and metabolites in dogs after subcutaneous administration of ceftiofur sodium. J Vet Pharmacol Ther 18:363–369

Brown SA, Deleeuw NR, Stahl GL, Roof RD (1995b) Characterization of plasma and lung concentrations after ceftiofur sodium and tilmicosin phosphate administered subcutaneously to mice. J Vet Pharmacol Ther 18:385–387

Brown SA, Chester ST, Robb EJ (1996) Effects of age on the pharmacokinetics of single dose ceftiofur sodium administered intramuscularly or intravenously to cattle. J Vet Pharmacol Ther 19:32–38

Brown SA, Hanson BJ, Mignot A, Millerioux L and others (1999) Comparison of plasma pharmacokinetics and bioavailability of ceftiofur sodium and ceftiofur hydrochloride in pigs after a single intramuscular injection. J Vet Pharmacol Ther 22:35–40

Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol 8: 1137–1144

Carli S, Perretta G, Brusa T, Invernizzi A, Faustini R (1983) Comparison of pharmacokinetics of sodium and lysine cephalexin in calves. J Vet Pharmacol Ther 6: 181–185

Carli S, Anfossi P, Villa R, Castellani G, Mengozzi G, Montesissa C (1999) Absorption kinetics and bioavailability of cephalexin in the dog after oral and intramuscular administration. J Vet Pharmacol Ther 22:308–313

Cunha BA, Gill MV (1995) Cefepime. Med Clin North Am 79:721–732

Day DN, Sparks JW, Karriker LA, Stalder KJ and others (2015) Impact of an experimental PRRSV and *Streptococcus suis* coinfection on the pharmacokinetics of ceftiofur hydrochloride after intramuscular injection in pigs. J Vet Pharmacol Ther 38:475–481

Dixon BA, Issvoran GS (1992) The activity of ceftiofur sodium for *Aeromonas* spp. isolated from ornamental fish. J Wildl Dis 28:453–456

Foster DM, Jacob ME, Warren CD, Papich MG (2016) Pharmacokinetics of enrofloxacin and ceftiofur in plasma, interstitial fluid, and gastrointestinal tract of calves after subcutaneous injection, and bactericidal impacts on representative enteric bacteria. J Vet Pharmacol Ther 39: 62–71

Fultz L, Giguere S, Berghaus LJ, Grover GS, Merritt DA (2015) Pulmonary pharmacokinetics of desfuroylceftiofur acetamide after nebulisation or intramuscular administration of ceftiofur sodium to weanling foals. Equine Vet J 47:473–477

Garg SK, Chaudhary RK, Srivastava AK, Garg BD (1990) Pharmacokinetics and dosage regimen of cephalexin in buffalo calves (*Bubalus bubalis*) following single intravenous and intramuscular administration. Vet Res Commun 14:59–62

Garg SK, Chaudhary RK, Srivastava AK (1996) Pharmacokinetics of cephalexin in calves after intravenous and subcutaneous administration. Acta Vet Hung 44:195–201

Garrett EF, Dirikolu L, Grover GS (2015) Milk and serum concentration of ceftiofur following intramammary infusion in goats. J Vet Pharmacol Ther 38:569–574

Gibaldi M, Perrier D (1982) Multicompartment models. In: Swarbrick J (ed) Pharmacokinetics, 2nd edn. Marcel Dekker, New York, NY, p 45–111

Giguère S, Sturgill TL, Berghaus LJ, Grover GS, Brown SA (2011) Effects of two methods of administration on the pharmacokinetics of ceftiofur crystalline free acid in horses. J Vet Pharmacol Ther 34:193–196

Goudah A (2007) Pharmacokinetics of ceftiofur after single intravenous and intramuscular administration in camels (*Camelus dromedarius*). J Vet Pharmacol Ther 30: 371–374

Grosset C, Weber ES III, Gehring R, Sanchez-Migallon Guzman D and others (2015) Evaluation of an extended-release formulation of ceftiofur crystalline-free acid in koi (*Cyprinus carpio*). J Vet Pharmacol Ther 38:606–615

Hektoen H, Bergea JA, Hormazabalb V, Yndestadb M (1995) Persistence of antibacterial agents in marine sediments. Aquaculture 133:175–184

Horsberg E (1994) Experimental methods for pharmacokinetic studies in salmonids. Annu Rev Fish Dis 4: 345–358

Jaglan PS, Roof RD, Yein FS, Arnold TS, Brown SA, Gilbertson TJ (1994) Concentration of ceftiofur metabolites in the plasma and lungs of horses following intramuscular treatment. J Vet Pharmacol Ther 17:24–30

Katharios P, Iliopoulou-Georgudaki J, Antimisiaris S, Verri T, Toma P, Acierno R, Maffia M (2004) Pharmacokinetics of cephalexin in sea bream, *Sparus aurata* (L.), after a single intraperitoneal injection. J Appl Ichthyology 20: 422–426

Lam FC, Hung CT, Perrier DG (1985) Estimation of variance for harmonic mean half-lives. J Pharm Sci 74:229–231

Maffia M, Verri T, Danieli A, Thamotharan M, Pastore M, Ahearn GA, Storelli C (1997) H(+)-glycyl-L-proline cotransport in brush-border membrane vesicles of eel (*Anguilla anguilla*) intestine. Am J Physiol 272:R217–R225

Martinsen B, Oppegaard H, Wichstrom R, Myhr E (1992) Temperature-dependent in vitro antimicrobial activity of four 4-quinolones and oxytetracycline against bacteria pathogenic to fish. Antimicrob Agents Chemother 36: 1738–1743

Rigos G, Nengas I, Alexis M, Troisi GM (2004) Potential drug (oxytetracycline and oxolinic acid) pollution from Mediterranean sparid fish farms. Aquat Toxicol 69: 281–288

Rivera-Garcia S, Angelos JA, Rowe JD, Byrne BA, Wetzlich SE, Van Liew DB, Tell LA (2014) Pharmacokinetics of ceftiofur crystalline-free acid following subcutaneous administration of a single dose to sheep. Am J Vet Res 75: 290–295

Robinson I, Junqua G, Van Coillie R, Thomas O (2007) Trends in the detection of pharmaceutical products, and their impact and mitigation in water and wastewater in North America. Anal Bioanal Chem 387:1143–1151

Silley P, Brewster G (1988) Kill kinetics of the cephalosporin antibiotics cephalexin and cefuroxime against bacteria of veterinary importance. Vet Rec 123:343–345

Smith CM, Reynard AM (1995) Essentials of pharmacology. WB Saunders, Philadelphia, PA

Soback S, Ziv G, Kurtz B, Paz R (1987) Clinical pharmacokinetics of five oral cephalosporins in calves. Res Vet Sci 43:166–172

Sohlberg S, Aulie A, Søli NE (1994) Temperature-dependent absorption and elimination of flumequine in rainbow trout (*Oncorhynchus mykiss* Walbaum) in fresh water. Aquaculture 119:1–10

Somjetlertcharoen A (2001) Evaluation of ceftiofur sodium as a chemotherapeutic agent in grass carp (*Ctenopharyn-*

godon idella). PhD dissertation, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA. http://scholar.lib.vt.edu/theses/available/etd-04052001-122730/ (accessed July 28, 2014)

Tell L, Harrenstien L, Wetzlich S, Needham M and others (1998) Pharmacokinetics of ceftiofur sodium in exotic and domestic avian species. J Vet Pharmacol Ther 21: 85–91

Thamotharan M, Gomme J, Zonno V, Maffia M, Storelli C, Ahearn GA (1996a) Electrogenic, proton-coupled, intestinal dipeptide transport in herbivorous and carnivorous teleosts. Am J Physiol 270:R939–R947

Thamotharan M, Zonno V, Storelli C, Ahearn GA (1996b) Basolateral dipeptide transport by the intestine of the teleost *Oreochromis mossambicus*. Am J Physiol 270: R948–R954

Verri T, Maffia M, Storelli C (1992) H^+/glycyl-glycine cotransport in eel intestinal brush-border membrane vesicles: studies with the pH-sensitive dye Acridine orange. Biochim Biophys Acta 1110:123–126

Verri T, Maffia M, Danieli A, Herget M, Wenzel U, Daniel H, Storelli C (2000) Characterisation of the H^+/peptide cotransporter of eel intestinal brush-border membranes. J Exp Biol 203:2991–3001

Villa R, Belloli C, Cagnardi P, Sonzogni O, Bacchetta S, Carli S (2002) Pharmacokinetics of cephalexin in the horse after intravenous and intramuscular administration of two formulations. Vet J 164:74–76

Yancey RJ Jr, Kinney ML, Roberts BJ, Goodenough KR, Hamel JC, Ford CW (1987) Ceftiofur sodium, a broad-spectrum cephalosporin: evaluation *in vitro* and *in vivo* in mice. Am J Vet Res 48:1050–1053

Editorial responsibility: Thomas Braunbeck, Heidelberg, Germany

Submitted: October 19, 2015; Accepted: June 13, 2016
Proofs received from author(s): August 6, 2016