**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 \( \beta \)-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.

---

**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 \( \beta \)-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 (NAX-CEL\textsuperscript{®}) 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 \( (C_{tot}) \) 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

---

© Inter-Research 2016 · www.int-res.com

© Inter-Research 2016 · www.int-res.com

2015), although other cephalosporins, such as cefqui-
ome and cephalexin, have been the objects of a
detailed pharmacokinetic study in salmon and sea
bream, respectively (San Martin et al. 1998, Kathar-
ios 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 effec-
tive 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 con-
trolling bacterial diseases in tilapia cultures, which
may result in environmental pollution with antibi-
otics through leaching of antibiotics from uneaten
fish feed or through tilapia excrements (Robinson et
al. 2007). This may result in adverse ecological ef-
fects, 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

The objective of this paper was to obtain informa-
tion 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 com-
mercially 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 equi-
valents 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 anes-
thesitized in benzocaine bath (100 mg l⁻¹ for 5 min expo-
sure) after being captured. Ceftiofur sodium was ad-
ministered 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 microbiolog-
ically 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 coeffi-
cient 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 pro-
gram 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:
\[ F = \left( \frac{AUC_{i.m.}}{AUC_{i.c.}} \right) \times 100 \]

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:

\[
\text{Conc.} = A \times \exp(-\alpha \times T) + B \times \exp(-\beta \times T) \tag{1}
\]

where \( T \) is time in hours, \( A \) and \( B \) are zero-time plasma concentration intercepts of the biphasic disposition curve, and \( \alpha \) and \( \beta \) 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:

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

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 \( k_{12}, k_{31} \), and the tissue-to-plasma-level ratio at the peak tissue level \( k_{12}/k_{31} \)). These findings significantly \((p \leq 0.05)\) prolonged the distribution \((t_{0.5(o)})\) of ceftiofur after intramuscular treatment \((0.49 \pm 0.06\) h vs. \(0.14 \pm 0.03\) h in i.c.-treated fishes).

The maximum concentrations \((C_{\text{max}})\) of 12.32 ± 6.53 µg ml\(^{-1}\) in plasma were reached after 0.74 ± 0.04 h (time of maximum concentration, \(t_{\text{max}}\)). 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 \(0.61 \pm 0.22\) 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 (24.75 ± 13.30 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 ($t_{0.5(ab)}$) 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>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>µg ml⁻¹</td>
<td>64.82 ± 27.31</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>h⁻¹</td>
<td>5.04 ± 1.01</td>
</tr>
<tr>
<td>$t_{0.5(ab)}$</td>
<td>h</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>$B$</td>
<td>µg ml⁻¹</td>
<td>11.47 ± 4.46</td>
</tr>
<tr>
<td>$\beta$</td>
<td>h⁻¹</td>
<td>1.13 ± 0.33</td>
</tr>
<tr>
<td>$t_{0.5(b)}$</td>
<td>h</td>
<td>0.61 ± 0.22</td>
</tr>
<tr>
<td>$k_{12}$</td>
<td>h⁻¹</td>
<td>1.12 ± 0.41</td>
</tr>
<tr>
<td>$k_{21}$</td>
<td>h⁻¹</td>
<td>1.83 ± 0.77</td>
</tr>
<tr>
<td>$K_{el}$</td>
<td>h⁻¹</td>
<td>3.22 ± 0.48</td>
</tr>
<tr>
<td>$Cl_{tot}$</td>
<td>l kg⁻¹ h⁻¹</td>
<td>1.64 ± 0.47</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>0.52 ± 0.07</td>
</tr>
<tr>
<td>Vdss</td>
<td>l kg⁻¹</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>AUC</td>
<td>µg ml⁻¹ h</td>
<td>24.18 ± 6.81</td>
</tr>
<tr>
<td>AUMC</td>
<td>µg ml⁻¹ h²</td>
<td>12.93 ± 6.18</td>
</tr>
</tbody>
</table>
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, distribution and excretion 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**


Editorial responsibility: Thomas Braunbeck, Heidelberg, Germany

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