Single intravenous and oral dose pharmacokinetics of florfenicol in the channel catfish (*Ictalurus punctatus*)

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**INTRODUCTION**

Florfenicol is a broad spectrum antibiotic in the phenicol class approved for use in a wide variety of fish species in 25 countries around the world. Florfenicol has proven efficacious in a wide variety of fish species against *Aeromonas salmonicida* (Inglis et al., 1991; Nordmo et al., 1994; Sheppard et al., 1994; Samuelsen et al. 1998), *Aeromonas* spp., (Luštek, 2002), *Vibrio salmonicida* (Nordmo et al., 1998), *Streptococcus iniae* (Bowker et al., 2010; Darwish, 2010; Gaunt et al., 2010b), *Photobacterium damselae* (Fukui et al., 1987), *Vibrio anguillarum* (Fukui et al., 1987; Samuelsen & Bergh, 2004), *Edwardsiella tarda* (Fukui et al., 1987), *Aeromonas hydrophila* (Fukui et al., 1987) and *Francisella asiatica* (Soto et al., 2010), *Flavobacterium psychrophilum* (Gultepe & Tansel Tanrikul, 2006), *Flavobacterium columnare* (Gaunt et al., 2010a,b), and *Edwardsiella ictaluri* (Gaunt et al., 2003,2004,2006).

Florfenicol was efficacious in channel catfish (*Ictalurus punctatus*) at a dose regimen of 10 mg/kg/day in feed for 10 days against the most commonly diagnosed catfish bacterial pathogens, *E. ictaluri* (the etiologic agent of enteric septicemia of catfish) (Gaunt et al., 2003, 2004, 2006) and *F. columnare* (the etiologic agent of columnaris disease) (Gaunt et al., 2010a). The pharmacokinetics of florfenicol reported for a variety of fish species (Reimischuessel et al., 2005) consistently demonstrate high bioavailability, high volume of distribution, and rapid elimination. However, the pharmacokinetics of florfenicol have not been measured in channel catfish, *Ictalurus punctatus*. We conducted this study to identify information that supports the florfenicol product labels for the control of mortality associated with enteric septicemia of catfish and columnaris disease in catfish. Herein, we report the single intravenous (i.v.) and oral dose pharmacokinetics for florfenicol in channel catfish administered at a dose of 10 mg/kg.

**MATERIALS AND METHODS**

**Animals**

Two hundred and ten healthy, 1–2-year-old channel catfish (*I. punctatus*) that weighed between 0.153 and 1.83 kg and with no prior history of exposure to pharmaceutical agents were seined from ponds on the Delta Research and Extension Center (Stoneville, MS, USA) for the study. Fish were brought to the wet laboratory at the Thad Cochran National Warmwater Aquaculture Center.

Individual fish were transferred to 60L flow-through glass aquaria, one fish/tank, and they were fed commercially extruded diet at 32% protein (Melick Aquafeed LLC, Greenville, MS, USA).
at ~1% bw/day. The fish were allowed to acclimate for at least 7 days prior to treatments. Throughout the studies, tank water was maintained at 25.4 °C ± 0.68 which is within the temperature range when outbreaks of enteric septicemia of catfish and columnaris disease occur (Noga, 2000). All results of water quality chemistry assays during these studies were within acceptable limits for the maintenance of catfish (Tucker & Hargreaves, 2004).

**Test articles and dose administration**

Florfenicol reference standard, deuterated florfenicol internal standard (d₄-florfenicol, 500 ng/mL), and Aquallor® Type A Medicated Article (50% florfenicol feed premix) were supplied by Intervet Inc. d/b/a Merck Animal Health, Summit, NJ, USA.

On the same day as the i.v. dose administration, the florfenicol reference standard was dissolved in propylene glycol and heated to 35 °C for 10 min to yield a nominal concentration of 10 mg FFC/mL propylene glycol. The single i.v. injection of florfenicol was delivered by a 21-gauge butterfly catheter in the caudal tail vein (Terumo Corp, Tokyo, Japan) to deliver 10 mg FFC/kg b.w. After injections, the catheter was flushed with a solution of 0.9% sodium chloride containing 10 U/mL heparin and then removed. A sample of the florfenicol dose solution was archived and stored at ~60 °C in microfuge tubes until assayed.

For the oral dose administration, commercial catfish feed containing 32% crude protein (Melick Aquafeed, LLC, Greenville, MS, USA) was ground and repelleted as previously described (Li et al., 1993). The lower limit of quantitation was 50 ng florfenicol/mL. Feed containing 20 g florfenicol/kg feed was prepared and then stored in a monitored refrigerator at 4 ± 2 °C 1 day prior to use. The single dose of medicated feed was mixed with the dry ground feed similarly to the commercial manufacturing process of preparing medicated feed and then extruded at ambient temperature as 8 inch diameter pellets. Feed extruded at ambient temperature as 1 inch pellets. Florfenicol medicated feed pellets were force fed from a 1 mL syringe in which the tip of the syringe was cut off and flame polished.

**Experimental design**

Animals were arbitrarily assigned to groups of 10 for each sample point and were fasted overnight before antibiotic administration. After treatment, each fish was transferred to a separate tank where it was observed for 5 min for adverse reactions or possible regurgitation of the test compound (orally dosed). If feed was regurgitated, the fish was removed from the study and replaced.

For the i.v. group, fish were sampled at predose, 0.166, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 36 h postdosing. For the oral group, fish were sampled at predose, 2, 4, 8, 10, 12, 24, 36, and 48 h postdosing. At the designated sampling time, 10 fish from each group were anesthetized by immersion in a bath of 300 mg/L tricaine methane sulfonate (MS-222) and ~4 mL blood samples were collected in EDTA tubes by caudal venipuncture. Following sampling, the fish were euthanized by an overdose in a solution of 300 mg/L MS 222. Plasma was collected after centrifugation and stored at ~60 °C in microfuge tubes until assayed.

**Pharmacokinetic and statistical analysis.** Individual florfenicol plasma concentration–time curves were analyzed using commercially available software. Intravenous data were fit to one-, two-, and three-compartment models. Oral data were fit to a one- or two-compartment extravascular models, each with and without a lag time. The model with the lowest Akaike Information Criterion was selected as the best fit. The florfenicol plasma concentrations of 10 fish at each sampling time were used as the basis for pharmacokinetic modeling. Standard pharmacokinetic parameters were calculated from the

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**Florfenicol in i.v. dose formulation and plasma**

**Plasma extraction/cleanup.** For the determination of the catfish plasma florfenicol concentrations, 100 μL aliquots of untreated control plasma, fortified control plasma, or plasma from treated fish were manually pipetted into the appropriate wells of a 96-well plate. After the addition of 100 μL of an aqueous internal standard solution, samples were acidified with 200 μL of 0.1 N hydrochloric acid and then underwent a solid phase extraction (SPE) cleanup step using Waters Oasis HLB SPE plates (10 mg) prior to LC-MS/MS analysis.

**Intravenous dose solution analysis.** The i.v. dose solution (10 mg/mL nominal concentration) was diluted and analyzed along with a solution of florfenicol analytical standard of a known concentration and determined to contain 10.1 mg/mL. Mean plasma concentrations for the i.v. dose were corrected for this difference from nominal.

**Instrumentation/LC-MS/MS analysis.** The plasma and i.v. dose solutions were analyzed by LC-MS/MS to determine florfenicol concentrations. Plasma sample extracts were analyzed in a negative ion mode on a Thermo Scientific Quantum Ultra System with Thermo Surveyor autosampler and pump. Flow rate was 800 μL/min with a 10 μL injection volume. Monitored transitions were from 356 to 185 m/z for fluorfenicol and from 360 to 189 m/z for d₄-florfenicol. A Phenomenex Synergi Hydro RP LC column (50 x 2.0 mm) was used for separation.

The lower limit of quantitation was 50 ng florfenicol/mL plasma, and the upper limit of quantitation was 5000 ng/mL. A matrix standard curve was used for quantitation and the curve fit was quadratic with 1/X² weighting. Florfenicol was stable in catfish plasma for 179 days stored at ~80 °C and subjected to three freeze/thaw cycles. Duplicate aliquots of control catfish plasma were fortified at 150, 2500, and 4500 ng/mL, and extracted along with each set of plasma samples from treated fish. The analytical method performance met requirements as stated for bioanalytical methods in the US-Food and Drug Administration Guidance for Industry 145 (May 2001).

**Pharmacokinetic and statistical analysis.** Individual florfenicol plasma concentration–time curves were analyzed using commercially available software. Intravenous data were fit to one-, two-, and three-compartment models. Oral data were fit to a one- or two-compartment extravascular models, each with and without a lag time. The model with the lowest Akaike Information Criterion was selected as the best fit. The florfenicol plasma concentrations of 10 fish at each sampling time were used as the basis for pharmacokinetic modeling. Standard pharmacokinetic parameters were calculated from the
relationship between plasma concentrations vs. time using the computer program MonoLix version 3.1R2 (Monolix, 2010); and WinSAAM version 3.0.7 (WinSAAM, Kennett Square, PA, USA).

RESULTS

LC-MS/MS plasma analysis

For the i.v. dose group, mean plasma florfenicol concentrations peaked at 10 min and declined rapidly thereafter. For the oral dose group, mean plasma florfenicol concentrations peaked at 9.2 h and declined thereafter.

Pharmacokinetic analysis

Data from the i.v. dose group best fit a two-compartment open model, and data from the oral dose groups fit a one-compartment open model with a lag time. The disposition curve for florfenicol i.v. and oral single dosing is presented in Fig. 1. The terminal half-life of florfenicol after i.v. injections was 8.25 h, volume of distribution at steady state ($V_{ss}$) was 0.9 L/kg, and the central volume of distribution was 0.381 ± 0.036 L/kg (Table 1). The mean peak plasma concentration of florfenicol at 9.2 h was 7.607 ± 0.867 µg/mL following oral administration. The oral mean half-life was 9.11 h. Oral bioavailability was 1.09.

DISCUSSION

Florfenicol exhibited similar pharmacokinetic properties in channel catfish as reported for other warmwater fish species (Yanong et al., 2005; Park et al., 2006; Feng et al., 2008). After a lag time of 1.67 h, orally administered florfenicol was absorbed from the catfish intestine in 9.2 h ($T_{max}$) with a maximum florfenicol plasma concentration ($C_{max}$) of 7.6 µg/mL. Lag time is defined as the delay between drug administration and its detection in blood. It corresponds to processes occurring during the absorption phase such as disintegration of medicated feed or negligible florfenicol absorption from the stomach with predominant absorption from the intestinal tract (Nerella et al., 1993). A lag time was not reported in a study with Korean catfish (Silurus asotus) which was conducted under similar warm, freshwater conditions (Park et al., 2006). In our study, florfenicol was incorporated into the feed which slows absorption of the medication until dissolution of the feed (Horsberg, 1994). Although the $T_{max}$ for the Korean catfish study was only 8 h (vs. 9.2 h for the channel catfish), no detail was provided on the formulation of the orally administered medicated feed (Park et al., 2006). If it were administered in solution for direct gavage or top dressed onto the feed, the medication would be more readily absorbed than if it were incorporated into feed pellets.

In spite of the absorption lag time, florfenicol appeared to be well absorbed from the intestine of channel catfish with an estimated bioavailability of 1.09. This is in agreement with the high bioavailability of florfenicol in other fish species, i.e. 0.92 in Korean catfish (Park et al., 2006) and 0.99 in Atlantic salmon (Salmo salar L.; Horsberg et al., 1996).

The high bioavailability of florfenicol contrasts with the other commercially available antimicrobials for catfish in the US. The bioavailability of oxytetracycline incorporated into catfish feed is <0.05 (Plakas et al., 1988) and the bioavailability of ormetoprim (one of the two active ingredients of Romet®) when incorporated in catfish feed catfish is 0.52 (Plakas et al., 1990).

Although we did not explore the elimination route of florfenicol in channel catfish in this study, florfenicol was eliminated by bile in freshwater-reared tilapia (Oreochromis niloticus; Bowser et al., 2009) and Korean catfish (Park et al., 2006). Bile excretion may result in reabsorption of drug and entero-hepatic cycling and to some extent retard drug elimination (Plakas et al., 1988; Bowser et al., 2009). This premise may partially account for the high bioavailability (>1.0) of florfenicol in our studies.

The elimination $T_{1/2}$ of florfenicol in channel catfish was 9.11 h for the oral group. This value was slightly shorter than
Table 1. Mean pharmacokinetic parameters (n = 10) for intravenous and oral definitive pharmacokinetic studies in channel catfish (Ictalurus punctatus) at mean water temperatures of 25.4 °C after dosing at 10 mg florfenicol/kg bw

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters (units)</th>
<th>Intravenous (mean ± SD)</th>
<th>Oral gavage (mean ± SD)</th>
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</thead>
<tbody>
<tr>
<td>T_{max} (h)</td>
<td>0.23 ± 0.14</td>
<td>9.20 ± 2.53</td>
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<tr>
<td>C_{max} (µg/mL)</td>
<td>22.31 ± 6.42</td>
<td>7.61 ± 0.87</td>
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<tr>
<td>V_{(L/kg)}</td>
<td>0.38 ± 0.04</td>
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<tr>
<td>V_{(l/kg)}</td>
<td>0.90 ± 0.13</td>
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<tr>
<td>Elimination t_{1/2} (h)</td>
<td>8.25</td>
<td>9.11</td>
</tr>
<tr>
<td>T_{lag} (h)</td>
<td></td>
<td>1.67 ± 0.42</td>
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<tr>
<td>AUC_{0–24} (h*µg/mL)</td>
<td>129.16 ± 6.95</td>
<td>128.75 ± 21.65</td>
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<tr>
<td>AUC_{inf} (h*µg/mL)</td>
<td>140.79 ± 6.46</td>
<td>138.92 ± 20.93</td>
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<tr>
<td>F</td>
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REFERENCES


