

Pharmacokinetics of florfenicol in the red pacu (*Piaractus brachypomus*) after single dose intramuscular administration

G. A. LEWBART*

M. G. PAPICH[†] &

D. WHITT-SMITH*

Departments of *Clinical Sciences and [†]Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

(Paper received 29 September 2004; accepted for publication 8 November 2004)

Dr. Gregory A. Lewbart, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA. E-mail: greg_lewbart@ncsu.edu

Florfenicol is a structural analogue of chloramphenicol similar to thiamphenicol, but with more activity against some bacteria than chloramphenicol (Cannon *et al.*, 1990). Florfenicol works by inhibiting bacterial protein synthesis at the ribosome (Cannon *et al.*, 1990). Florfenicol activity against bacteria differs from chloramphenicol because florfenicol is not susceptible to the same resistance mechanisms as chloramphenicol (Papich & Riviere, 2001). Organisms resistant to chloramphenicol may still be susceptible to florfenicol. Florfenicol has been demonstrated to be efficacious against bacteria (e.g. *Aeromonas salmonicida*, *Vibrio salmonicida*, and *Edwardsiella ictaluri*) of fish, especially salmonids and catfish (Fukui *et al.*, 1987; Inglis & Richards, 1991; Inglis *et al.*, 1991; Nordmo *et al.*, 1994, 1998; Samuelson *et al.*, 1998; Gaunt *et al.*, 2003, 2004). Florfenicol has been proven to be clinically effective in controlling a variety of bacterial diseases in salmonids and is approved for use in Europe, Norway, Canada, Japan, and South Korea for a variety of fish species (Gaunt *et al.*, 2003).

Florfenicol has been administered orally for treatment of bacterial infections of captive fish in Europe and Canada under the trade names Aquafer[®] and Aquaflor[®] (Schering-Plough, Kenilworth, NJ, USA), respectively. In rainbow trout (*Oncorhynchus mykiss*) kept at 10 °C, an oral dose of 10 mg/kg had a mean residence time of 21 h and a C_{max} of 3.23 mcg/mL (Pinault *et al.*, 1997). Pharmacokinetics have also been described for Atlantic salmon, *Salmo salar* (Martinsen *et al.*, 1993). Other work has determined florfenicol to be safe and effective for the treatment of *E. ictaluri* (enteric septicemia of catfish) in channel catfish (*Ictalurus punctatus*) when administered orally in feed (Gaunt *et al.*, 2003, 2004).

The objectives of this study were to determine the maximum serum concentrations, elimination half-life, and relative bioavailability of florfenicol in the red pacu following single-dose intramuscular (i.m.) administration.

We used 16 red pacu for the study. Fish were of uniform age and size (approximately 3.5 years old and weighing 400–500 g each). Fish were individually maintained in sixteen 75 L

aquariums which all shared a common water supply via a recirculating system. Important water quality parameters such as temperature (25 °C), pH 7.2, total alkalinity (51.0 mg/L), and specific gravity (1.000) were frequently monitored and actively maintained.

Twelve fish were weighed immediately prior to epaxial i.m. dosing with 10.0 mg/kg florfenicol (NuFlor[®], Schering-Plough). The four control fish received equivalent volumes of i.m. saline. Fish were manually restrained and approximately 0.4 mL of blood was taken from the caudal vein from each fish and four control fish using a sodium heparinized 1 cc syringe with a 25 G needle at the following times postdrug administration: 0, 3, 4, 6, 9, 12, 24, 48, and 72 h. The four control fish were used to determine if any residual or excreted florfenicol in the recirculating system was absorbed by the study pacu.

Florfenicol plasma concentrations were analyzed with reverse-phase high performance liquid chromatography (HPLC). The HPLC apparatus consisted of a pump (Waters Model 600 Pump; Millipore Corp., Milford, MA, USA), autosampler (Hewlett Packard Series 1050 Autosampler; Hewlett Packard, Palo Alto, CA, USA), UV detector (HP Series 1050 UV detector; Hewlett Packard), and computer for data collection and analysis (Hewlett Packard HPLC^{2D} ChemStation running Windows 3.1 on a Hewlett Packard Vectra 486/33N computer). Eluates were separated with a C-8 reverse-phase HPLC column (Zorbax RX-C8 4.6 mm × 15 cm; 5 µm MAC-MOD Analytical Inc., Chadds Ford, PA, USA). A guard column containing identical packing material also was used (Zorbax RX-C18, 4 mm × 1.25 cm guard column).

Florfenicol was eluted with a mobile phase consisting of 73% distilled water and 27% (v/v) acetonitrile. No buffers or mobile phase modifiers were added. The mobile phase was filtered and degassed prior to use and was continuously sparged with helium during the analysis. The flow rate was 1.0 mL/min. Injection volume was 20 µL. Florfenicol was detected with UV detection at a wavelength of 223 nm. Retention time for florfenicol was approximately 5.5–6.5 min.

A stock solution of 1 mg/mL were prepared by dissolving a pure reference standard in acetonitrile. The stock solution was kept refrigerated in a tightly sealed vial. The stock solution was then diluted serially with distilled water to make spiking solutions ranging from 1.000 to 1.95 mcg/mL. Control (blank) plasma was obtained from untreated animals. A volume of 20 mL of the spiking solutions were added to 180 mL of blank plasma to produce 10 calibration standards ranging from 100 to 0.195 mcg/mL. A blank sample also was analyzed with each batch of samples every day.

A new calibration curve was prepared for each day's samples. Approximately 24 samples were analyzed each day. In order for the calibration curve to be accepted, it had to be linear with an r^2 value of at least 0.99 and the calibration standards had to be back-calculated to within 15% of the true value. Unknown concentrations were calculated by plotting the concentrations of florfenicol against response value (peak height). Concentration of study samples was calculated from the response value (Fig. 1).

The samples from the study, as well as the prepared calibration plasma samples, were prepared by pipetting 100 mL into a clean screw-top glass tube. To this tube 100 mL of phosphate buffer was added. Phosphate buffer was prepared by adding 13.6 g of monobasic potassium phosphate to 1.0 L of distilled water. The pH of the buffer was adjusted to 7.0.

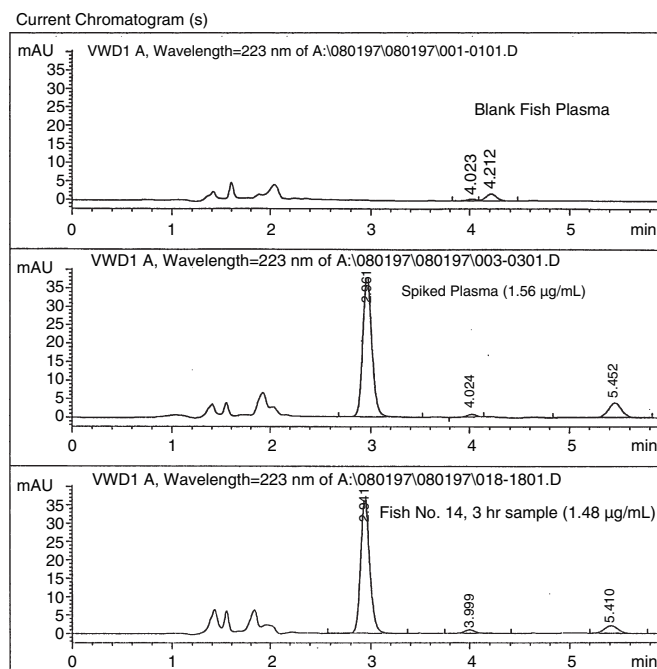


Figure 1. Sample HPLC chromatogram from study. Thiamfenicol (internal standard) and florfenicol peaks in chromatogram had retention times of approximately 2.9 and 5.4 min, respectively. Top panel: chromatogram from blank (untreated) fish plasma. Middle panel: chromatogram from bank fish plasma spiked with thiamfenicol (internal standard) and florfenicol (spiked concentration 1.56 mcg/mL). Bottom panel: chromatogram from fish taken from actual study (fish no. 14 at 3 h after injection). Calculated concentration in sample was 1.48 mcg/mL.

After brief vortexing, 2 mL of ethyl acetate was added and the mixture was gently rocked for 10 min at room temperature.

The tubes were centrifuged at 550 *g* for 10 min at -6°C . An amount of 1 mL of the supernatant was transferred to another clean glass tube and the contents evaporated under a flow of nitrogen (20 psi) at 45°C for 15 min. The residue in each tube was reconstituted with 200 mL of the mobile phase and briefly vortexed and transferred to an HPLC vial for injection.

Plasma concentrations of florfenicol were plotted on a semi-logarithmic graph for analysis. The following parameters for disposition of florfenicol were calculated from plasma concentration vs. time curves: first-order elimination rate constant (k_{el}), y -axis intercept (C_0), terminal half-life ($t_{1/2}$), area-under-the-curve from time zero to infinity (AUC), volume of distribution area method (Vd_{area}/F), and total systemic clearance (Cl_s/F). These calculations were made using methods described by Gibaldi and Perrier (1982). Note that values for volume of distribution and clearance are corrected for systemic availability (F) because the dose was administered i.m. Values for the elimination rate constant and intercept of the curves were calculated with the use of a nonlinear curve fitting program (WinNonlin; SCI Scientific Consulting Inc., Cary, NC, USA).

The HPLC analysis for florfenicol was rapid with a high degree of reproducibility. Calibration curves had an r^2 value of >0.99 . The precision of the assay was within 6.9 and 7.6% of the mean at the low and high concentration, respectively. Accuracy, reported as the percent of deviation from the true value was 1.26 and 2.5% for the low and high concentration, respectively. The limit of quantitation was approximately 0.39 mcg/mL. Concentrations below this value were not used for pharmacokinetic analysis.

Pharmacokinetic analysis could not be performed on fish no. 1 because of too few data points above the limit of quantitation. For the other 11 experimental fish, the mean peak plasma concentration at 3 h was 1.09 mcg/mL (± 0.12). The mean half-life, Vd/F , and Cl/F was 4.25 h, 5.69 L/kg, and 0.92 L/kg/h, respectively. Plasma concentrations are shown in the graph (Fig. 2). Table 1 contains a summary of the study's raw data. There were no detectable levels of florfenicol in the plasma of the control fish or the time zero samples of the experimental fish.

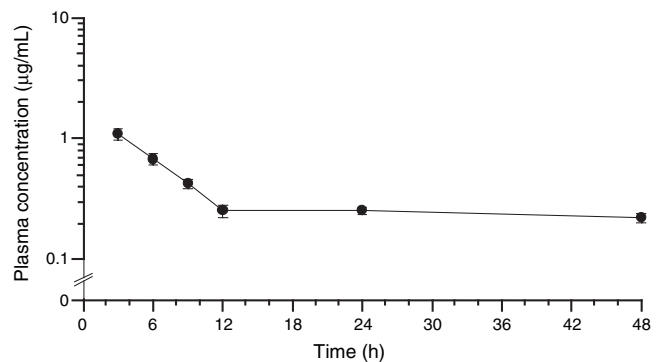


Figure 2. Mean plasma concentrations over time of florfenicol in red pacu following i.m. administration of florfenicol at a dose of 10.0 mg/kg body weight.

Table 1. Pharmacokinetic parameters for 12 experimental and four control red pacu after 10.0 mg/kg i.m. administration of Florfenicol

| Parameter | Value | Units | Standard deviation |
|---|-------|------------------|--------------------|
| Apparent volume of distribution (VD/F)* | 5.69 | L/kg | 2.3 |
| Elimination rate | 0.17 | 1/h | 0.05 |
| Elimination half-life ($T_{1/2}$) | 4.25 | h | 1.02 |
| Systemic clearance (Cl/F)* | 0.92 | L/kg/h | 0.29 |
| Peak concentration (C_{max}) at 3 h | 1.09 | $\mu\text{g/mL}$ | 0.12 |

*Values for volume of distribution and clearance are listed as corrected for systemic absorption (F). True values are not known because there was no i.v. drug administration.

These data show that florfenicol was eliminated rapidly and had a large volume of distribution when injected i.m. in red pacu maintained at 25 °C at a dose of 10 mg/kg. While this study does not address a specific bacterial pathogen, the existence of *in vitro* MIC data supports the statement that significant plasma concentrations of florfenicol can be obtained in the red pacu after a single 10 mg/kg i.m. injection. The 3 h C_{max} of 1.09 mcg/mL just exceeds the reported 0.8 mcg/mL *in vitro* MIC for *A. salmonicida*, *Vibrio anguillarum*, and *V. salmonicida* (Martinsen *et al.*, 1993). The MIC for *E. ictaluri* was determined to be 0.25 mcg/mL (McGinnis *et al.*, 2003). It is entirely possible that the actual red pacu florfenicol C_{max} was substantially higher prior to the 3 h time point, but our data collecting intervals, based on salmonid protocols (Martinsen *et al.*, 1993; Pinault *et al.*, 1997), likely missed the peak plasma concentration.

Further work is needed to determine the true C_{max} , establish an accurate dosing regimen, and ascertain the safety of florfenicol in pacu and other ornamental fish. Based on the data presented in this paper, the authors suggest a dose of 20–30 mg/kg i.m. every 24 h in order to obtain plasma values of florfenicol that exceed the MIC for *Aeromonas* and *Vibrio* species.

ACKNOWLEDGMENTS

The authors would like to thank Delta Dise for assistance with sample analysis and Jessica Geyer for assisting with fish maintenance. This study was supported by the state of North Carolina.

REFERENCES

Cannon, M., Jarford, S. & Davies, J. (1990) A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some

fluorinated analogs. *Journal of Antimicrobial Chemotherapy*, **18**, 311–316.

- Fukui, H., Fujihara, Y. & Kano, T. (1987) *In vitro* and *in vivo* antibacterial activities of florfenicol, a new fluorinated analog of thiamphenicol, against fish pathogens. *Fish Pathology*, **22**, 201–207.
- Gaunt, P., Endris, R., Khoo, L., Leard, T., Jack, S., Santucci, T., Katz, T., Radecki, S.V. & Simmons, R. (2003) Preliminary assessment of the tolerance and efficacy of florfenicol against *Edwardsiella ictaluri* administered in feed to channel catfish. *Journal of Aquatic Animal Health*, **15**, 239–247.
- Gaunt, P.S., Endris, R.G., Khoo, L., Howard, R., McGinnis, A.L., Santucci, T.D. & Katz, T. (2004) Determination of dose rate of florfenicol in feed for control of mortality in channel catfish *Ictalurus punctatus* (Rafinesque) infected with *Edwardsiella ictaluri*, etiological agent of enteric septicemia. *Journal of the World Aquaculture Society*, **35**, 257–267.
- Gibaldi, M. & Perrier, D. (1982) *Pharmacokinetics*, 2nd edn. Marcel Dekker, New York.
- Inglis, V. & Richards, R.H. (1991) The *in vitro* susceptibility of *Aeromonas salmonicida* and other fish pathogenic bacteria to 29 antimicrobial agents. *Journal of Fish Disease*, **14**, 641–650.
- Inglis, V., Richards, R.H., Varma, K.J., Sutherland, I. & Brokken, E.S. (1991) Florfenicol in Atlantic salmon, *Salmo salar* L., parr: tolerance and assessment of efficacy against furunculosis. *Journal of Fish Disease*, **14**, 343–351.
- Martinsen, B., Horsberg, T.E., Varma, K.J. & Sams, R. (1993) Single dose pharmacokinetic study of florfenicol in Atlantic salmon (*Salmo salar*) in seawater at 11 °C. *Aquaculture*, **112**, 1–11.
- McGinnis, A., Gaunt, P., Santucci, T., Simmons, R. & Endris, R. (2003) *In vitro* evaluation of the susceptibility of *Edwardsiella ictaluri*, etiological agent of enteric septicemia in channel catfish, *Ictalurus punctatus* (Rafinesque), to florfenicol. *Journal of Veterinary Diagnosis Investigation*, **15**, 576–579.
- Nordmo, R., Varma, K.J., Sutherland, I.H. & Brokken, E.S. (1994) Florfenicol in Atlantic salmon, *Salmo salar* L.: field evaluation of efficacy against furunculosis in Norway. *Journal of Fish Diseases*, **17**, 239–244.
- Nordmo, R., Riseth, J.M.H., Varma, K.J., Sutherland, I.H. & Brokken, E.S. (1998) Evaluation of florfenicol in Atlantic salmon, *Salmo salar* L.: efficacy against furunculosis due to *Aeromonas salmonicida* and cold water vibriosis due to *Vibrio salmonicida*. *Journal of Fish Diseases*, **21**, 289–297.
- Papich, M.G. & Riviere, J.E. (2001) Chloramphenicol and derivatives, macrolides, lincosamides, and miscellaneous antimicrobials. In *Veterinary Pharmacology and Therapeutics*, 8th edn. Ed. Adams, H.R. pp. 868–897. Iowa State University Press, Ames, IA.
- Pinault, L.P., Millot, L.K. & Sanders, P.J. (1997) Absolute oral bioavailability and residues of florfenicol in the rainbow trout (*Onchorhynchus mykiss*). *Journal of Veterinary Pharmacology and Therapeutics*, **20** (Suppl. 1), 297–298.
- Samuelsen, O.B., Hjeltnes, B. & Glette, J. (1998) Efficacy of orally administered florfenicol in the treatment of furunculosis in Atlantic salmon. *Journal of Aquatic Animal Health*, **10**, 56–61.