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Single dose pharmacokinetic study of florfenicol in Atlantic salmon (*Salmo salar*) in seawater at 11°C

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ABSTRACT

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The pharmacokinetics of intravenously and orally administered florfenicol were determined in Atlantic salmon (*Salmo salar*) weighing 194 ± 40 g (mean ± s.d.). The study was performed at 10.8 ± 1.5°C. A dose of 10 mg florfenicol/kg body weight was administered either intravenously or orally to groups of 85 fish each. At seven time points, from 3 h to 120 h after administration, blood was sampled from 10 individual fish in each group. The plasma was assayed for florfenicol using an HPLC method. The pharmacokinetic modelling of the results was performed using the computer program PCNONLIN.

Following intravenous administration, the plasma concentration–time data of florfenicol were best described by a two-compartment open model. The volume of distribution at steady state, $V_{d(ss)}$, and the total body clearance, Cl_T, were 1.122 l/kg and 0.086 l/h·kg, respectively. The elimination halflife, $t_{1/2}\beta$, was estimated as 12.2 h. Following oral administration, the plasma concentration–time data of florfenicol were best described by a one-compartment open model with first order absorption and elimination. Peak plasma concentration, C_{max}, was estimated at 4.0 μg/ml and was estimated to occur at 10.3 h (T_{max}) following dosing. The bioavailability, F, was estimated at 96.5%.

Based on the median minimum inhibitory concentrations (MICs) of 0.8 μg/ml reported for *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio salmonicida*, plasma concentrations should remain above the MIC for 36–40 h following a single oral dose of 10 mg florfenicol/kg.

INTRODUCTION

Substantial development of resistance against quinolones, sulphonamide/trimethoprim combinations and tetracyclines in fish-pathogenic bacteria (Tsoumas et al., 1989; Barnes et al., 1990; Inglis et al., 1991b; Høie et al.,

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1992) has led to a need for new antimicrobial drugs for the control of bacterial diseases in fish. In addition, enhanced bioavailability of new agents compared to those currently in use may reduce the environmental impact of antibacterial drugs.

Florfenicol, a broad spectrum antibacterial agent, is a fluorinated derivative of thiamphenicol, a chloramphenicol analogue in which the *p*-nitro group on the aromatic ring is substituted with a sulfonylmethyl group (Fig. 1). Chloramphenicol analogues lacking this nitro group have not been linked to aplastic anaemia (Krishna et al., 1981), and hence, are potential antimicrobial drugs for food-producing animals including fish.

Florfenicol has shown equal or superior in vitro activity compared to thiamphenicol and chloramphenicol against bacteria pathogenic to man (Neu and Fu, 1980; Syriopoulou et al., 1981). In addition, bacteria resistant to chloramphenicol and thiamphenicol due to production of acetyltransferase are susceptible to florfenicol (Neu and Fu, 1980; Syriopoulou et al., 1981).

In vitro studies with florfenicol have shown potent activity against several fish pathogenic bacterial species, including *Aeromonas salmonicida* and *Vibrio anguillarum* (Fukui et al., 1987; Inglis and Richards, 1991). Similar results were observed for florfenicol against *A. salmonicida* subsp. *salmonicida*, *V. anguillarum* and *Vibrio salmonicida* (Y. Torgersen, 1992, National Veterinary Institute, Oslo, personal communication).

Florfenicol has also shown therapeutic efficacy against experimentally induced pseudotuberculosis (*Pasteurella piscicida*) in yellowtail (*Seriola quinqueradiata*) (Fukui et al., 1987; Yasunaga and Yasumoto, 1988), *Edwardsiella tarda* infection in eel (*Anguilla japonica*) (Fukui et al., 1987), vibriosis (*V. anguillarum*) in goldfish (*Carassius auratus*) (Fukui et al., 1987), and furunculosis (*A. salmonicida*) in Atlantic salmon (*Salmo salar*) parr (Inglis et al., 1991a).

Information concerning the pharmacokinetic properties of florfenicol in

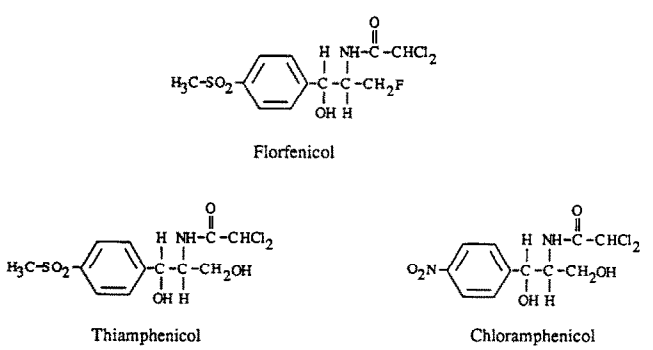

Fig. 1. The structures of florfenicol, thiamphenicol, and chloramphenicol.

Atlantic salmon is, however, not available. The aims of this study were to examine the pharmacokinetic properties of florfenicol in Atlantic salmon in seawater after intravascular and oral administration, and to calculate the bioavailability based on the pharmacokinetic parameters.

MATERIALS AND METHODS

Test substance

Florfenicol was obtained from Schering-Plough Corporation, Kenilworth, NJ, USA. The substance for intravenous administration was dissolved in propylene glycol (10 mg/ml). The florfenicol for oral administration was mixed into ordinary fish feed by Ewos Aquaculture A/B, Södertälje, Sweden, at a concentration of 2 g florfenicol/kg feed. The test feed was mixed, pelleted and heat extruded at 85–90°C for 15–20 s. Analysis of the test feed showed a concentration of 1.95 g florfenicol/kg feed.

Experimental design

The study was conducted at NIVA Marine Research Station, Solbergstrand, Drøbak, Norway. The fish were held in fibre glass tanks of 1 m^3 capacity supplied with running seawater with a salinity of about 30‰. The study was performed at 10.8 ± 1.5°C.

Two hundred experimental fish were obtained from Marenor A/S, Slemmestad, Norway. The fish were Atlantic salmon postsmolts of the Svanøy strain, weighing 194 ± 40 g (mean ± s.d.).

The fish were randomly divided into two groups of 100 each 14 days prior to initiation of the study, and transferred to the test tanks for adaptation. They were fed a commercial pelleted fish diet (Vextra, Ewos) ad libitum once a day during the adaptation period. On the day prior to drug administration and during the experimental period, the fish were not fed.

One group of fish was given florfenicol intravenously at a dose of 10 mg/kg. The other group was given florfenicol orally as medicated feed supplying a dose of 10 mg/kg. Eighty-five fish in each group were administered the drug.

In the group given florfenicol intravenously, each fish was netted from the adaptation tank and weighed in a small tank of water placed on a Mettler PC 16 balance. After weighing, each fish was anaesthetized with chlorbutanol (300 mg/l water). The intravenous injection was accomplished by placing the fish dorsally on damp paper in a V-formed tray. The florfenicol solution was slowly injected into the caudal vein using a 1 ml disposable syringe and a 23G×1 needle (Terumo, Belgium). The injection volume was 1.0 ml/kg body weight. The position of the needle in the caudal vein was confirmed by aspirating blood into the syringe prior to, during and following the injection. If the needle had translocated during the injection, the fish was excluded and replaced.

In the group administered florfenicol orally, each fish was netted from the tank and weighed. Florfenicol medicated feed corresponding to 10 mg florfenicol per kg body weight, was then weighed on a Sartorius L 420 P balance and loaded in a 1 ml disposable syringe (Terumo, Belgium) in which the delivery part previously had been cut off. The syringe was inserted down the oesophagus, and the medicated feed deposited in the stomach by depressing the plunger through the syringe. During administration, each fish was manually restrained without anaesthesia. Following administration of the test feed, each fish was transferred to a small individual tank containing about 8 l of water and observed for possible regurgitation over a period of approximately 5 min. Fish which regurgitated feed pellets were excluded from the study and replaced.

Ten fish from each group were killed at each time point by a blow to the head. Blood samples were taken from each fish by caudal venipuncture using 21G×1.5 needles and heparinized vacutainers (5 ml) (Venoject™, Terumo Europe N.V., Leuven, Belgium). Blood samples from fish given the drug i.v. were collected at least 2 cm away from the injection site. Blood was sampled at the following times: before drug administration, and 3, 6, 12, 24, 48, 72 and 120 h post-administration. After centrifugation at 3000 rpm for 10 min, the plasma was collected and frozen at −20°C in plastic vials until analyzed. Each fish in the oral group was examined after blood sampling for rupture of the stomach.

Method of analysis

The concentration of florfenicol in plasma was determined by means of high performance liquid chromatography (HPLC). The system consisted of a dual piston reciprocating pump (Consta Metric III G, LDC/Milton Roy, Riviera Beach, FL, USA), autosampler (WISP model 712 B, Waters, Division of Millipore, Milford, MA, USA), variable wavelength ultraviolet absorption detector (Spectroflow model 773, Applied Biosystems, Ramsey, NJ, USA) operated at 223 nm, and reporting integrator (model 4270, Varian Associates, Walnut Creek, CA, USA). The chromatographic column was a 250 mm×4.6 mm i.d. stainless steel column packed with 5-μm particle size reverse-phase chromatography medium (Nucleosil C18, Alltech Associates, Arlington Heights, IL, USA). A 20 mm×2 mm i.d. guard column (Uptight, Upchurch Scientific, Oak Harbor, WA, USA) packed with 10-μm particle size reverse-phase chromatography medium (Ultrapack ODS, Beckman Instruments, Inc., Fullerton, CA, USA) was attached to the chromatographic column. The mobile phase was 35% acetonitrile in water at a flow rate of 1.0 ml/min. Chromatographic analyses were performed at ambient temperature.

Sample treatment

To 1.5-ml Eppendorf microcentrifuge tubes was added 4.0 μl of the thiamphenicol stock solution (1.0 μg/ml) for use as the internal standard. To each

of these tubes was added a 0.50-ml aliquot of plasma (calibrator, quality control sample, or unknown sample). Each sample was mixed and 0.50 ml of acetonitrile was added to each tube to precipitate proteins. The tubes were vortex-mixed for 10 s and microcentrifuged for 3 min. The aqueous supernatant solutions were decanted into 16×100 mm glass tubes. One ml of 0.10 *M* phosphate buffer (pH 7.00) was added to each, and after mixing, 4.0 ml of ethyl acetate was added to each tube. The tubes were mixed by end-over-end rotation for 10 min to extract the florfenicol and then centrifuged at 2200×*g* for 10 min to separate the phases. The supernatant solutions were transferred to 13×100 mm glass tubes and evaporated at 45°C under a stream of nitrogen. After evaporation, the sides of each tube were rinsed with approximately 0.5 ml of methylene chloride which was then evaporated as before. The residues were dissolved in 500 μl of mobile phase solution, sonicated for 2 min to ensure complete dissolution, and then transferred to 1.2-ml autosampler vials. Calibration concentrations were 0.1, 0.3, 0.6, 1, 3, 6, and 10 μg/ml. Quality control samples were 0.1 and 6 μg/ml.

The plasma concentration of florfenicol was determined by comparison with standards prepared in florfenicol-free plasma. The lower limit of quantitation of florfenicol in Atlantic salmon plasma was approximately 0.1 μg/ml.

Pharmacokinetic analysis

In the group given florfenicol intravenously, standard pharmacokinetic parameters were calculated from the best fitted relationship between mean plasma concentration and time according to a two-compartment open model (Baggot, 1977) using the pharmacokinetic computer program PCNONLIN, version 01-H. A one-compartment open model was used to calculate pharmacokinetic parameters following oral administration.

RESULTS

The mean florfenicol concentrations in ten fish at each sampling time were used as the basis for pharmacokinetic modelling. Mean plasma florfenicol concentrations are given in Table 1.

Data from the intravenously dosed group were best described by a two-compartment open model with bolus i.v. administration and first-order elimination. Data from the orally dosed group were best described by a one-compartment open model with first-order input and first-order output, with no lag time. The observed plasma-concentration versus time curve in both the intravenous and the oral group are presented in Fig. 2.

The time point of maximum plasma concentration of florfenicol following oral administration, T_{max}, was estimated as 10.3 h and the maximum plasma concentration, C_{max}, was estimated as 4.0 μg/ml. Following i.v. administration the apparent volume of distribution at steady state, $V_{d(ss)}$, was estimated

TABLE 1

Mean florfenicol concentrations in plasma of Atlantic salmon (*Salmon salar*) following a single i.v. or single oral administration of florfenicol (10 mg/kg). Salmon were kept at 10.8 ± 1.5 °C in running sea water

Time after administration (h)	Number of sampled fish	Mean plasma florfenicol, concentration ± s.e.m.	
		i.v. administered (μg/ml)	orally administered (μg/ml)
0.	10	0.00	0.00
3	10	7.44 ± 0.33	1.60 ± 0.21
6	10	5.24 ± 0.15	3.75 ± 0.20
12	10	2.96 ± 0.11	4.41 ± 0.11
24	10	1.33 ± 0.11	2.15 ± 0.13
48	10	0.30 ± 0.08	0.36 ± 0.04
72	10	<0.10*	<0.10
120	10	<0.10	<0.10

*Limit of detection of 0.1 μg/ml.

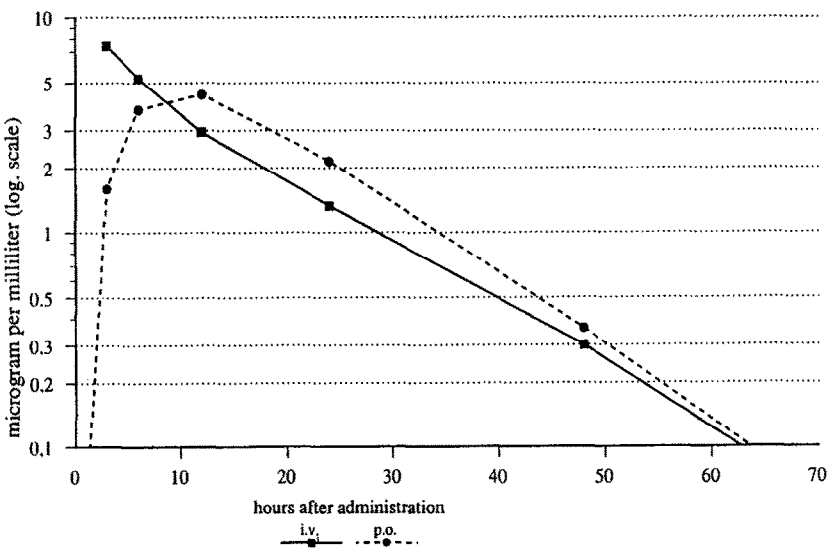

Fig. 2. The mean plasma concentration–time profile in Atlantic salmon following intravenous or oral administration of 10 mg florfenicol/kg. (Limit of detection of 0.1 μg/ml.)

to be 1.122 l/kg and the total body clearance, Cl_T, was 0.086 l/h·kg. The elimination halflife, $t_{1/2}\beta$, was estimated as 12.2 h. The bioavailability, F, was estimated at 96.5%. All estimated parameters are listed in Table 2.

In the intravenously dosed group, a certain degree of hyperactivity lasting

TABLE 2

Pharmacokinetic parameters calculated in Atlantic salmon held in running sea water at 10.8°C, following a single dose of 10 mg florfenicol/kg intravenously or orally

Parameter	i.v. administration	p.o. administration
A	6.2 μg/ml	
B	5.0 μg/ml	
$V_{d(ss)}$	1.122 l/kg	
Cl_T	0.086 l/h·kg	
α	0.22 h^{-1}	
β	0.06 h^{-1}	
$t_{1/2\beta}$	12.2 h	
AUC	116.3 h·μg/ml	112.0 h·μg/ml
C_{max}		4.0 μg/ml
T_{max}		10.3 h
F		96.5%

approximately 2 h (increased jumping activity, distress) was observed 1–2 h after administration. No adverse effects were noted in the orally dosed group.

DISCUSSION

The adverse reaction observed in the intravenously administered group (1–2 h after injection) was believed to be caused by the propylene glycol used as a vehicle for florfenicol. In vitro, propylene glycol is known to result in progressive swelling of human red cells followed by lysis (Demey et al., 1988), and there are also reports of acute haemolysis following intravenous administration of propylene glycol used as a drug vehicle in humans (Demey et al., 1988). In sheep, intravenous injection of propylene glycol caused haemoglobinuria (Potter, 1958).

In blood samples collected 3 and 6 h post i.v. administration, a considerable degree of haemolysis was noticed. For samples obtained at 12 h or later in the i.v. administered fish and from the orally administered fish, no haemolysis was seen. There may be a connection between the propylene glycol used as a vehicle, the hyperactivity, and the haemolysis seen in the i.v. administered fish. However, there is no evidence that the haemolysis had any effect on the pharmacokinetics of intravenously administered florfenicol.

The absorption of florfenicol following administration of florfenicol-medicated heat-extruded feed pellets was fairly rapid. The highest plasma concentration, 4.0 μg/ml (C_{max}), occurred at 10.3 h (T_{max}) after administration. Both C_{max} and T_{max} may be altered by the oral drug vehicle used (Hustvedt et al., 1991b), co-administration of feed (Hustvedt et al., 1991a,b), and by the method of incorporation of the drug into feed. If the test compound is coated on the outside of the feed pellet, the time to reach T_{max} would be ex-

pected to be shorter compared to a drug formulation where the test compound is incorporated within the pellet.

The bioavailability, F, for florfenicol in Atlantic salmon held in seawater was estimated at 96.5%, which is higher than the bioavailability of 88% observed in veal calves (Varma et al., 1986). The bioavailability of florfenicol was higher than for other antimicrobial drugs currently used to treat bacterial infections in Atlantic salmon. For example, the bioavailability of oxolinic acid ranges from 13.6 to 38.1% depending on dose, fish species, salinity and water temperature (Cravedi et al., 1987; Björklund, 1991; Hustvedt et al., 1991b), while oxytetracycline and tetracycline show an even lower bioavailability ranging from 0.6 to 8.6% depending on food intake, fish species and water quality parameters (Cravedi et al., 1987; Grondel et al., 1987; Plakas et al., 1988; Björklund, 1991). Bowser et al. (1992) reported a 25% bioavailability of enrofloxacin in fingerling rainbow trout in freshwater at 10°C. The use of drugs possessing high bioavailability in fish should be an important consideration towards reducing the environmental impact of therapeutic drugs. Dose rates may be reduced and the waste of unabsorbed drugs minimized.

An apparent volume of distribution of 1.122 l/kg indicates that florfenicol is well distributed throughout the body, suggesting that tissue concentrations would be similar to those obtained in plasma.

The total body clearance after i.v. administration was calculated at 0.086 l/h·kg, which is higher than the Cl_T of other antibacterial agents currently in use. Björklund (1991) reported a Cl_T of 0.016 and 0.020 l/h·kg in rainbow trout at 16°C in freshwater for oxytetracycline and oxolinic acid, respectively. In Atlantic salmon held at 9°C in seawater, a Cl_T of 0.03 l/h·kg was reported for oxolinic acid (Hustvedt et al., 1991a), while Cl_T of 0.05 and 0.083 l/h·kg was reported for oxolinic acid in rainbow trout at 8.5°C in freshwater and seawater, respectively (Hustvedt and Salte, 1991).

The elimination of florfenicol was rather rapid, as the elimination halflife, $t_{1/2}\beta$, was calculated to be 12.2 h. This is shorter than the $t_{1/2}\beta$ of other antibacterial agents used in aquaculture. In freshwater a $t_{1/2}\beta$ of 6.1 days was reported for oxytetracycline in rainbow trout at 10°C (Björklund and Bylund, 1990), while oxolinic acid had a $t_{1/2}\beta$ of about 4 days in rainbow trout at 10°C (Björklund et al., 1992). Hustvedt and Salte (1991) reported a $t_{1/2}\beta$ of 52.6 h and 29.1 h at 8.5°C for oxolinic acid in rainbow trout in freshwater and seawater, respectively. The shorter elimination halflife of oxolinic acid in seawater was confirmed by Ishida (1992). In Atlantic salmon held at 9°C in seawater, halflives ranging from 37.1 to 60.3 h were reported for oxolinic acid (Hustvedt et al., 1991a).

The rapid total body clearance and the short biological halflife of florfenicol suggest that its withdrawal period in Atlantic salmon in seawater might be shorter than for other drugs currently in use. However, neither the influence of water temperature on excretion nor the presence of metabolites of florfen-

icol was investigated in the present study. In addition, the method used for assaying the samples had a limit of detection of 0.1 μg/ml; hence, low concentrations of residues originating from tissue binding cannot be ruled out. Such binding is known to exist for several antibacterial agents. For instance, tetracyclines bind to bone and cartilage (Ingebrigtsen et al., 1985), and trimethoprim binds to melanin-containing tissues (Bergsjø et al., 1979).

Designing an effective dose regimen for florfenicol in Atlantic salmon requires knowledge of florfenicol pharmacokinetics and the sensitivity of fish pathogens to the agent. The sensitivity of several bacterial species pathogenic to Atlantic salmon has been examined. Minimum inhibitory concentrations (MICs) from 0.3 to 1.6 μg/ml have been reported for *A. salmonicida* and *V. anguillarum* (Fukui et al., 1987; Inglis and Richards, 1991; Y. Torgersen, 1992, personal communication). Torgersen (pers. commun., 1992) also examined ten strains of *V. salmonicida*, which all showed MICs of 0.8 μg/ml. All strains tested, except for *Y. ruckeri*, had MICs for florfenicol equal to or less than 1.6 μg/ml, and the majority of these strains had MICs equal to or less than 0.8 μg/ml. The calculated $C_{max} = 4.0$ μg/ml for florfenicol at a dose of 10 mg/kg was 5 times the median MIC of 0.8 μg/ml reported for *A. salmonicida*, *V. anguillarum* and *V. salmonicida* (Fukui et al., 1987; Inglis and Richards, 1991; Y. Torgersen, pers. commun., 1992). The MICs against ten *Y. ruckeri* strains reported by Y. Torgersen (pers. commun.) ranged from 6.4 to 17.1 μg/ml, while MICs against five strains described by Inglis and Richards (1991) ranged from 0.6 to 10.0 μg/ml. Hence, florfenicol may be less effective in the treatment of *Y. ruckeri* infections due to the lower susceptibility of this species.

Resistance of fish-pathogenic bacteria to quinolones, potentiated sulphonamides and tetracyclines is becoming an increasing problem in aquaculture (Tsoumas et al., 1989; Barnes et al., 1990; Inglis et al., 1991b; Høie et al., 1992). Antibacterial agents such as florfenicol with alternative modes of action may, in the future, be of great value in the control of bacterial diseases in fish.

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