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Excretion of flumequine in free-swimming Atlantic salmon (*Salmo salar*), determined by cannulation of the dorsal aorta, gall bladder and urethra

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Pharmacokinetics deals with the processes of drug absorption, distribution, metabolism and excretion. Studies in this field are normally done in individual animals. Due to the technical difficulties involved in establishing the necessary experimental methods, studies on the fate of aquaculture drugs and environmental pollutants have rarely been performed in individual fish. It has been customary to collect pharmacokinetic information from fish by giving drugs intravascularly or orally to a large group of fish, and taking blood samples by killing several fish at each sampling time point. However, a dorsal aorta cannulation technique has been used in several studies (Sohlberg *et al.*, 1990, 1994, 1996) to investigate the pharmacokinetic profile of flumequine in fish, by collecting blood samples repeatedly from individual free swimming fish. This technique may be supplemented with other procedures in order to better describe the disposition of xenobiotics in fish. Techniques allowing urine and bile to be collected from individual free swimming fish, may provide additional information about the route of drug excretion in fish. The aim of the present work was to study the excretion of flumequine in Atlantic salmon in sea water by combining various cannulation techniques, making it possible to collect blood, urine and bile samples repeatedly from individual free-swimming fish.

This study was conducted at VESO Vikan AkvaVet, Namsos, Norway. The fish and the experimental conditions were the same as described in Sohlberg *et al.* (1997). Immediately after surgery, which lasted for about 45 min, each fish was transferred separately to small specially designed 25 L metabolism tanks (Kleinow, 1991) (Fig. 1), and left undisturbed for 24 h. Before flumequine was given intravascularly, the flow of sea water into each metabolism tank was shut off, the water in the tanks thereafter being aerated by an air pump. The fish were otherwise kept under the same environmental conditions as during the acclimatization period.

The dorsal aorta cannulation technique developed by Smith & Bell (1964) and modified by Soivio *et al.* (1972, 1975) and Sohlberg *et al.* (1990, 1994, 1996) was used. Gall bladder cannulation was performed according to the method described by Sohlberg *et al.* (1997), while urethral cannulation was carried out using the technique by Beyenbach & Kirschner (1975) as modified by Kleinow (1991) (Fig. 1). Flumequine for intra-arterial administration was obtained from Unibios Control Laboratory (Alpharma A/S, Oslo, Norway). A solution was prepared by dissolving the drug in 0.1 M NaOH. The pH was then adjusted to 11.0 with HCl, before final volume adjustment with saline, giving concentrations of 5 mg/mL, 10 mg/mL and 25 mg/mL, respectively. The flumequine solution was administered to the fish 24 h after surgery *via* the cannula in the dorsal aorta at dosages of 5 mg/kg, 10 mg/kg and 25 mg/kg body weight (Table 1).

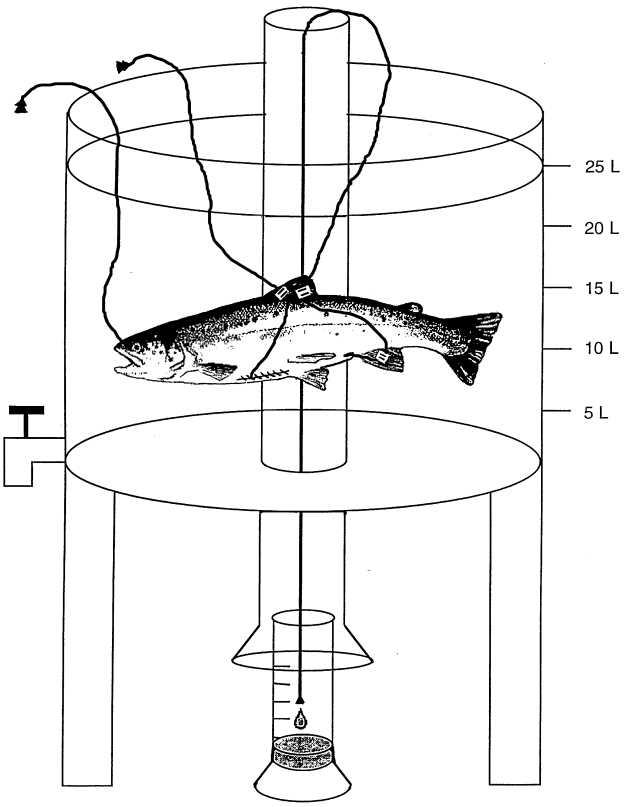

Fig. 1. Aortic cannula, urinary catheter and gall bladder catheter tending system for mass balance studies in free-swimming fish.

Table 1. Recovery of flumequine (%) in bile, urine and sea water in the metabolism chamber and whole body tissue after intravascular administration of flumequine. Fish no. 1–4 were given 10 mg/kg, fish no. 5, 5 mg/kg, and fish no. 6, 25 mg/kg

Fish no.	Fish weight (g)	Flumequine dose (mg/kg)	Sampling period (h)	Bile (%)	Urine (%)	Sea water (%)	Whole fish (%)	Recovered amount (%)
1	1013	10	72	0.48	0.19	64.27	3.11	68.1
2	912	10	72	0.52	6.35	60.31	4.16	71.3
3	977	10	72	0.83	0.26	64.93	0.73	66.8
4	1068	10	96	1.31	0.22	44.78	0.76	47.1
5	764	5	96	1.62	2.51	70.34	2.75	77.2
6	832	25	60	5.24	0.55	36.18	27.70	69.7

Blood, bile and urine samples were collected from all the fish before the drug was administered, and water samples were taken from the tanks before drug administration. Blood, bile and urine samples were subsequently collected 12, 24, 36, 48, 60 and 72 h after drug administration. Samples were also collected from some of the fish at 6, 84 and 96 h after drug administration (Table 2). In order to investigate the excretion of flumequine *via* the gills, 5 mL sea-water samples were collected from the tank at the same time as the urine and bile samples were taken. After each sampling 15 L of the water in the tank (25 L in all) were replaced with 15 L of fresh sea water. All the fish were starved during the experiment and hardly any faeces was found in the tanks. Nevertheless, small amounts of faeces were collected at 24, 48 and 72 h after flumequine administration from one fish. All the samples were put on ice immediately after sampling and stored in a freezer (-20 °C) until analysed. Immediately after samples were taken for the last time, which varied from 72 to 96 h after drug administration, the fish were killed by a blow to the head, and transferred without delay to a freezer (-20 °C), where they were stored until analysed. The plasma, urine, faeces and sea-water samples were cleaned up by solid phase extraction on a column of the Bond Elut® type, (Varian sample preparation products, Harbor City, CA, USA) size 1 cc, with C2 sorbent material, according to Rasmussen *et al.* (1989). The bile samples were cleaned up according to Hormazábal & Yndestad (1994). The whole fish were homogenized and whole body tissue samples cleaned up according to Steffenak *et al.* (1991). The concentrations of flumequine in plasma, bile, urine and water and whole body tissue were determined by means of high performance liquid chromatography (HPLC) using a fluorescence detector. The extracted components, flumequine, and the internal standard, were separated on a polymer column (PLRP-S, 5 μm; Polymer Laboratories, Shropshire, UK). The mobile phase was 0.02 M phosphoric acid: acetonitrile:tetrahydrofurane 62:18:20, delivered at a rate of 0.7 mL/min. The detector was operated at an excitation wavelength of 325 nm and emission wavelength of 360 nm. The limit of quantification was 10 ng/mL for all analytical methods.

Twenty fish were cannulated in the dorsal aorta, the gall bladder and the urethra. Six fish died during surgery because of damage to either the liver or to the vena porta hepatica; damage at either site led to excessive bleeding. This was mainly attributed to organ adhesion due to prior vaccination, with an associated high risk of haemorrhage and major or minor damage to the liver. Four fish were excluded during surgery because of rupture to the gall bladder wall. No fish were excluded because of complications associated with cannulation of the dorsal aorta and urethra, but four fish died 2–4 days after surgery for unknown reasons. It would be a great advantage to use unvaccinated fish in investigations which include cannulation of the gall bladder. The rate of secreted urine ranged from $\approx$ 0.2–0.8 mL/h/kg (Table 2). Talbot *et al.* (1989) found the normal urinary secretion rate in Atlantic salmon to be 0.72 mL/h/kg. The urine flow rate in salmonids in fresh water has been shown to increase immediately after anaesthesia, surgery and handling, normalizing 24 h later (Houston & DeWilde, 1969; Hunn & Willford, 1970; Hunn, 1982; Kleinow, 1991). Starvation of salmonids causes a decrease in urinary flow rate (Hunn, 1982). In the present study, sampling was not started until 24 h after surgery. Moreover the fish were starved, so that the lower urine flow rate we found in some fish may have been due to starvation. The rate of secreted bile sampled at each time point ranged from 0.15 to 0.76 μL/min/kg, and decreased in some fish during the experiment (Table 2). The little information concerning bile production rate in fish available to us was for dogfish and skates. Boyer *et al.* (1976) reported bile production rates in these species of 0.55–1.2 and 1.4–1.8 μL/min/kg, respectively. Sanz *et al.* (1993) cannulated the bile duct in trout and collected bile entering a bag fastened to the skin in the mid ventral line. They estimated the bile flow rate to be $\approx$ 2.9 μL/min/kg, after sampling for 24 h. Avery *et al.* (1992), found the flow rate of bile in fasted and fed trout to be 0.87 ± 0.18 μL/min/kg, and 2.1 ± 0.8 μL/min/kg, respectively. The gall bladder serves as a reservoir for bile, and we could not ascertain if all the bile contained in the gall bladder was in fact collected on each sampling occasion in our experiment. It is likely that the values found for the rate of bile production in this study are underestimations of the actual basal bile flow rate in this species, because of the previous fasting period. Nor can we exclude the possibility that the decrease in bile volume in some of the fish was caused by interruption of the enterohepatic circulation of bile salts, as we did not supply fresh bile into the gut *via* an indwelling stomach tube as done by Kleinow (1991).

The total amount of flumequine excreted as flumequine in percentage of the given dose is presented in Table 1. Most of the fish excreted more than 60% of the administered flumequine into the water (Table 1). However, fish no. 6,

Table 2. Flow rates, urine (mL) and bile (μL) volumes sampled from 6 fish after intravascular administration of flumequine to Atlantic salmon

Hours after administration	Urine sampling volumes (mL) 6	12	24	36	48	60	72	84	96	Flow rate (mL/h/kg)
Fish no.										
1	0.4	0.05	0.8	3.4	1.8	2.4	6.0	–	–	0.20
2	1.2	1.9	9.9	8.9	10.5	9.4	11.0	–	–	0.80
3	1.3	1.9	6.7	1.7	19.0	0.1	9.0	–	–	0.56
4	–	3.0	2.8	3.0	2.2	2.8	1.8	2.7	0.1	0.20
5	–	1.2	0.1	2.9	4.3	3.2	4.4	3.4	2.0	0.21
6	–	2.2	0.7	8.1	1.0	2.4	0.0	–	–	0.24

Hours after administration	Bile sampling volumes (μL) 6	12	24	36	48	60	72	84	96	Flow rate μL/min/kg
Fish no.										
1	160	160	70	70	48	80	80	–	–	0.15
2	80	10	90	20	170	120	110	–	–	0.15
3	110	440	240	0	20	30	80	–	–	0.22
4	–	310	310	300	310	330	490	430	540	0.49
5	–	270	260	320	20	70	80	140	130	0.29
6	–	760	620	620	190	110	420	–	–	0.76

which was given flumequine at a dosage of 25 mg/kg, excreted only 36% of the given dose into the water, but excreted about 5 times more flumequine into the bile than the other fish. The flumequine residue level found in this fish was higher (%) than in the other fish, the sample containing about 28% of the administered flumequine.

After each sampling, 15 L of the sea water in the metabolism tank (25 L in all) was replaced with fresh sea water. No fish seemed to be affected by this, all fish were allowed to swim freely throughout the experiment. The additional small amounts of faeces (0.1–2 g during the experiment) excreted by one fish into the metabolism tank are considered to make only a minor contribution to the total flumequine concentration in the 25 L of sea water. Nevertheless, it would be an advantage if faeces could be collected individually to avoid mixing of faecal and gill excretion. The flumequine concentrations in faeces were much higher than in the surrounding water. This is probably a result of an ion-trapping of flumequine in the gut, due to the higher pH of the intestinal content compared to the blood (Griffiths *et al.*, 1994). We found significant individual differences in the proportion of flumequine excreted by the various routes. Nevertheless, we were able to recover 66.7 ± 10.3% (SEM) of the total administered dose as flumequine in all the fish. We assume that flumequine metabolites probably explain part of the unrecovered amount of administered flumequine.

The application of the techniques to cannulate the gall bladder, urethra and the dorsal aorta in individual free swimming fish may provide very useful data about the elimination of drugs, other xenobiotics and their metabolites in fish. However, as the relative amounts of flumequine excreted *via* the urine, bile and the gills vary significantly between individual fish, at least 5–10 fish should be included in each group in such studies to compensate for this variation. Nevertheless the combination of these techniques may contribute to limit the amount of fish used for pharmacokinetic studies in the future.

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