Flumequine in Atlantic salmon *Salmo salar*: disposition in fish held in sea water versus fresh water

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ABSTRACT: ^{14}C-labeled flumequine was administered as a single oral (5 mg kg^{-1}, 86 µCi kg^{-1}) or intravenous (5 mg kg^{-1}, 82 µCi kg^{-1}) dose to Atlantic salmon *Salmo salar* held in sea water or in fresh water. The absorption, tissue distribution and elimination were determined by means of liquid scintillation counting and whole-body autoradiography. The drug was rapidly absorbed and extensively distributed in all groups of fish. Radiolabeled compound was present in blood and muscle for more than 8 wk in the freshwater groups. In the seawater groups, however, no radioactivity was detected in the blood and muscle after 4 d and 2 wk, respectively. It was concluded that flumequine was eliminated at a substantially higher rate from Atlantic salmon in sea water than in fresh water.

KEY WORDS: Flumequine · Kinetics · Whole-body autoradiography · Atlantic salmon

INTRODUCTION

Flumequine is a broad spectrum synthetic antimicrobial agent belonging to the 4-quinolones and is commonly used in aquaculture. The pharmacokinetic properties of flumequine have been evaluated in rainbow trout (Sohlberg et al. 1990, 1994), Atlantic salmon (Martinsen & Horsberg 1995, Sohlberg et al. 1996, 1997, 1999), turbot, halibut (Hansen & Horsberg 1999), eel (Hansen & Horsberg 2000a, Hansen et al. 2001), cod and goldsinny wrasse (Hansen & Horsberg 2000b) using different study designs. Previous investigations in fish provide evidence for different pharmacokinetic properties of quinolones in fresh water compared with sea water (Ishida 1992, Elston et al. 1994).

The purpose of the present study was to compare the disposition of flumequine after its intravenous (i.v.) and oral (p.o.) administration to Atlantic salmon *Salmo salar* held in fresh water with that in sea water.

MATERIALS AND METHODS

Test substance. ^{14}C-labeled flumequine was produced in 1995 by Dupont NEN Research Products with a radiochemical purity of 99%. When retested before the study, the purity was only 92%. It was therefore purified using a C2 solid-phase extraction column of the Bond Elut type to a radiochemical purity of >98%.

Experimental design. The freshwater study was conducted at The National Veterinary Institute, Oslo, Norway. The fish were kept in fiber glass tanks (240 l) supplied with running fresh water (pH 6.5, Ca: 2.75 mg l^{-1}, Mg: 0.48 mg l^{-1}), which was maintained at 11.9 ± 0.3°C during the experiment.

The seawater study was conducted at NIVA Marine Research Station, Solbergstrand, Drøbak, Norway. The fish were kept in fiber glass tanks (600 l) supplied with running sea water (pH 8, salinity 34‰), which was kept at 11.1 ± 1.1°C during the experiment. All the tanks were placed under controlled light conditions, corresponding to a photoperiod of 12:12 h light:dark.

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Table 1. *Salmo salar*. Mean amounts of flumequine (mg kg^{-1}) in organs (n = 2) from Atlantic salmon in fresh water (FW) and sea water (SW) after oral (p.o.) and intravenous (i.v.) administration of 82 and 86 µCi ^{14}C-labeled flumequine kg^{-1} (5 mg kg^{-1}), respectively. ND: non-detectable levels; NO: no observation

Hours	Muscle i.v.		Brain i.v.		Blood i.v.		Kidney i.v.		Liver i.v.		Bile i.v.		Skin i.v.	
	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW
6	3.36	1.01	2.59	1.17	2.25	1.39	6.27	2.74	4.41	2.38	1.34	169.1	3.95	4.4
24	1.25	0.35	1.07	0.55	1	0.44	1.7	1.07	2.41	1.01	NO	163.1	3.27	1.91
96	0.34	ND	0.32	0.01	0.23	0.02	0.57	0.06	0.57	0.03	13.57	25.32	1.16	0.13
336	0.05	0.01	0.84	0.01	0.05	ND	0.07	0.03	0.02	0.02	NO	2.29	0.07	0.02
1512	0.09	NO	0.07	0.01	0.09	NO	0.02	0.09	0.16	0.02	0.18	NO	0.02	0.01

Hours	Muscle p.o.		Brain p.o.		Bood p.o.		Kidney p.o.		Liver p.o.		Bile p.o.		Skin p.o.	
	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW	FW	SW
6	1.48	0.29	1.2	0.64	1.16	0.55	1.43	1.16	2.27	0.98	5.68	44.8	2.02	1.15
12	1.09	0.64	0.55	0.74	0.73	1.57	1.66	1.84	1.73	4.28	NO	11.09	1.91	2.08
24	0.95	0.08	0.73	0.17	0.68	0.11	1.36	0.37	2.09	0.23	18.82	12.78	2.09	0.44
48	0.59	0.02	0.61	0.01	0.89	0.01	0.57	0.03	0.73	0.03	14.11	4.06	1.3	0.08
96	0.23	ND	0.23	NO	0.18	ND	0.61	0.02	0.57	0.01	6.84	6.11	1.2	0.04
168	0.09	ND	0.75	0.01	0.11	ND	0.32	0.01	0.23	0.02	8.27	3.19	0.45	0.01
336	0.02	ND	0.05	0.01	0.02	ND	0.07	0.01	0.02	0.1	0.07	0.79	0.07	0.01
672	0.02	ND	0.2	ND	0.02	ND	0.11	ND	0.07	0.02	3.18	NO	0.11	0.01
1344	0.02	NO	0.43	NO	0.27	NO	0.05	NO	0.02	NO	0.05	NO	0.02	NO
1512	ND	NO	0.02	NO	0.02	NO	ND	NO	0.02	NO	0.05	NO	0.02	NO

Atlantic salmon *Salmo salar* with a mean ± SD weight of 37 ± 10 g in the freshwater group and 71 ± 32 g in the seawater group (Table 1) were both administered 86 µCi kg^{-1} p.o. or 82 µCi kg^{-1} i.v. of ^{14}C-labeled flumequine, corresponding to 5 mg flumequine kg^{-1} in each group after addition of cold flumequine. A stock solution was prepared by dissolving 8.65 mg ^{14}C-labeled flumequine and 41.34 mg cold flumequine in 2 ml 1 N NaOH. The flumequine for i.v. administration was diluted in saline, pH 10, and the injection volume was 2 ml kg^{-1} body weight. For p.o. administration flumequine was diluted in corn oil.

During the adaptation period the fish were fed ad libitum a commercial pelleted fish diet using automatic feeders. Feed was withheld 1 d prior to drug administration and feeding was resumed the following day.

In the group given i.v. flumequine solution, each fish was netted from the acclimation tank and individually weighed in a small tank of water. The fish was then anesthetized with benzocaine (50 mg l^{-1} water) for 3 to 4 min. The i.v. injection of flumequine was accomplished by placing the fish dorsally on damp paper in a V-form tray; the flumequine solution was slowly injected into the caudal vein using a 1 ml disposable syringe and a 23G × 1″ needle (Terumo).

In the group given p.o. flumequine, each fish was netted from the acclimation tank and weighed. The drug suspension was whirlmixed for 15 s and administered through a stomach tube (catheter no. 12, Rush), using an attached 1 ml disposable syringe. Each fish was manually restrained without anesthesia. After drug administration, all p.o. dosed fish were transferred to individual tanks for observation of regurgitation before being transferred to the experimental tanks. No regurgitation was observed; therefore, no fish were excluded.

A discard permit for the study was obtained from the Institute of Radiation Hygiene in Norway. To minimize the effluents of ^{14}C, the outlet water from the fish tanks was filtrated and all solid effluents were removed.

Sampling. In the i.v. administered groups, samples were collected at 6, 24, 96, 336 and 1512 h post administration. In the p.o. administered groups samples were collected at 6, 12, 24, 48, 96, 168, 336, 672, 1344 and 1512 h post administration. At each sampling time 2 fish were sacrificed by an overdose of benzocaine (200 mg l^{-1} for 10 min) and frozen in liquid nitrogen (–196°C). They were then embedded in individual blocks of cooled 1% solution of sodium carboxymethyl cellulose in water (0°C) followed by immediate freezing with dry ice in n-hexane (–75°C).

Analytical procedures. Each sample was analyzed using whole-body autoradiography and liquid scintillation spectrometry to detect radioactivity (Ullberg 1977). Sagittal sections (20 to 40 µm) from the whole fish were obtained on tape (No. 821, 3M) at –20°C in a PMV cryomicrotome (PMV; 450 MP). The sections were freeze dried over night, before application on Hyperfilm-βmax (Amersham) or Structurix D7 (Agfa) for autoradiography. The films were frozen at –20°C for approximately 3 mo before development.

From the material remaining in the frozen blocks, samples weighing 10 to 100 mg were obtained from muscle, brain, blood, kidney, liver, bile and skin. The samples were digested with 1 ml Soluene (Packard) at 37°C overnight. Ten milliliters of liquid scintillation cocktail (Hionic Fluor, Packard) was then added to each vial. The radioactivity in the samples was counted in a Packard Tri-Carb 1900CA liquid scintillation analyzer, using a Packard automatic quenching standard to estimate the counting efficiency.

RESULTS

Fresh water study

The concentration of flumequine equivalents (radioactivity) in the blood at 24 h was less than half the 6 h value and more than half the 6 h value after i.v. and p.o. administration, respectively (Fig. 1). Thereafter the concentration of radioactivity decreased gradually, and from 2 to 9 wk (1512 h) only minor changes were observed in both groups.

Following both p.o. and i.v. administration, flumequine was generally well distributed to all major tissues and organs. In the p.o. group no large differences in radioactivity levels between muscle, brain, blood and kidney samples were observed (Table 1). Higher levels were found in the liver and in the skin, while the highest levels of radioactivity were found in the bile. The radioactivity levels were higher in the i.v. group than in the p.o. group throughout the experiment, and high levels of radioactivity were observed in some of the samples from brain tissue after both p.o. and i.v. administration (Table 1).

Generally, the results from the autoradiographic study agreed well with those from the liquid scintillation counting study. Twenty-four hours after administration, the autoradiograms showed that flumequine was well distributed to all major tissues and organs
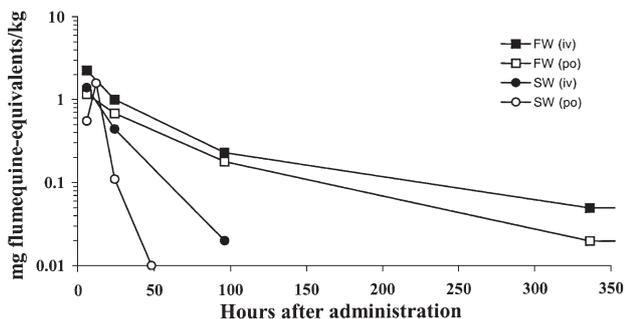

Fig. 1. *Salmo salar.* Flumequine equivalents in the blood of Atlantic salmon after oral (p.o.) (n = 2) and intravenous (i.v.) (n = 2) administration in sea water (SW) and fresh water (FW)

(Fig. 2A). At 4 d, radiolabeled compound was still present in most tissues and organs (Fig. 2B). The autoradiographic recordings also revealed a high and persistent level of radiolabeling in the skin, the uveal tract of the eye and the bone. Nine weeks after p.o. administration, there were still detectable amounts of radioactivity in bone (Fig. 2C).

Sea water study

Compared with the freshwater study, the concentrations of flumequine equivalents in all tissues and organs decreased more rapidly in fish held in sea water (Table 1). The concentration of flumequine equivalents in the blood at 24 h was nearly one-third of the value at 6 h, and traces were detected in the blood 4 d after the i.v. administration (Fig. 1). In the p.o. group the flumequine radioactivity concentration decreased more rapidly than was observed after i.v. administration, and only minor amounts of flumequine radioactivity were observed in the blood 48 h after administration.

The distribution pattern of flumequine and its metabolites, as demonstrated by autoradiography at 24 h (Fig. 3A) and 4 d (Fig. 3B) after p.o. administration, is in accordance with the results from the liquid scintillation determination.

DISCUSSION

The study was designed in accordance with the discard permit regulations from the Institute of Radiation Hygiene in Norway, which was the reason for the low number of fish included in the study. The main objective was to study the qualitative disposition of radiolabeled flumequine administered to fish in sea water versus fresh water, by observing the differences in the autoradiograms of the fish and the scintillation countings from samples taken at several time points after medication. Consequently, pharmacokinetic parameters were not calculated. Despite this methodological limitation, valuable information on the disposition of flumequine in Atlantic salmon was obtained. Unless otherwise specified, the following discussion refers to the p.o. dosed fish, which is the administration route of practical relevance.

The rapid and extensive distribution of flumequine in Atlantic salmon in this study was in accordance with previous reports of high distribution volumes of flumequine in other fish species (Martinsen & Horsberg 1995, Samuelsen & Ervik 1997, Hansen & Horsberg 1999, 2000a,b, Plakas et al. 2000). The time at which maximum plasma concentrations occurred (T_{max}) in this study was apparently reached within 6 h or less
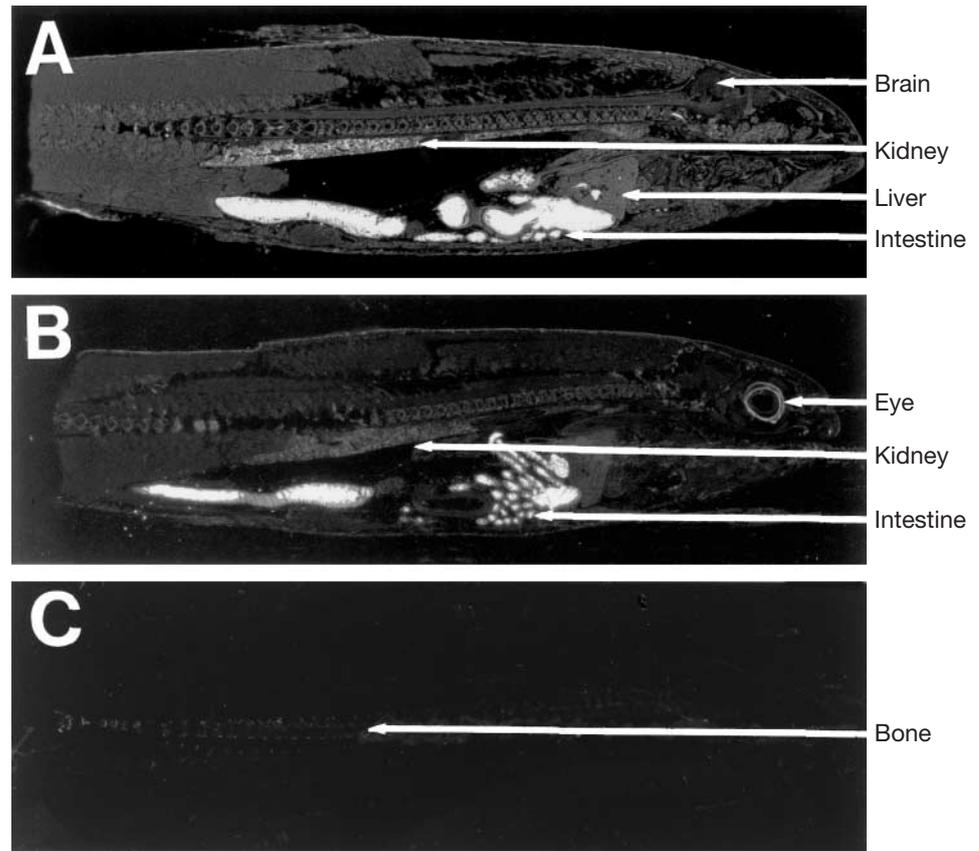
Fig. 2. *Salmo salar.* Distribution pattern of flumequine and its metabolites (A) 24 h, (B) 4 d and (C) 9 wk after p.o. administration to Atlantic salmon in fresh water
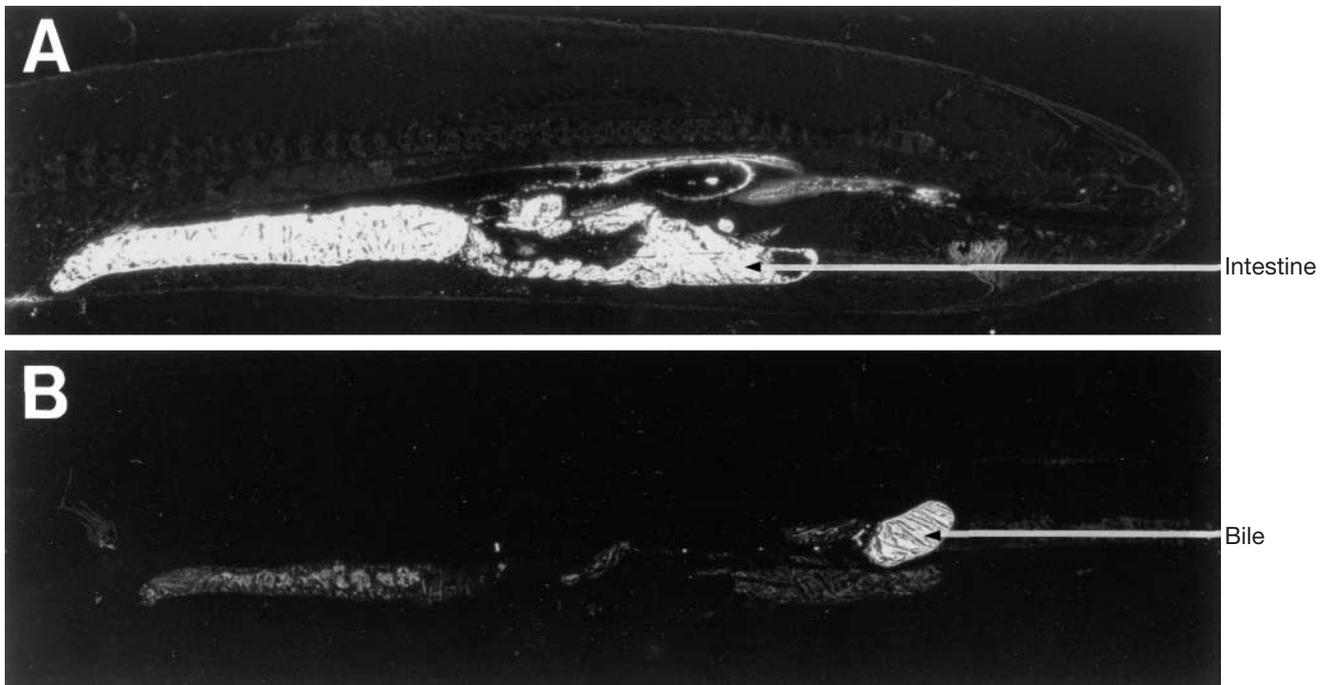

Fig. 3. *Salmo salar.* Distribution pattern of flumequine and its metabolites (A) 24 h and (B) 4 d after p.o. administration to Atlantic salmon in sea water

after p.o. administration, which corresponded well with T_{max} values of 2 to 12 h for flumequine administered to eels (Van der Heijden 1994). Furthermore, Hansen & Horsberg (1999) reported a T_{max} of 7 h for flumequine given to turbot and halibut.

The gills are regarded as one of the most important sites of xenobiotic elimination in fish, and they seem to be the main route by which fish excrete flumequine, as more than 60% of the flumequine administered to salmon in sea water was eliminated by the gills (Sohlberg et al. 1999). However, biliary excretion generally contributes considerably to drug elimination from fish, and the present study confirmed that the biliary route also is an important excretory pathway for flumequine in Atlantic salmon.

The faster elimination of flumequine in sea water than in fresh water may be explained by the influence of chelation on the compound's pharmacokinetic properties (Stein 1996). Sea water fish drink actively, and the chelation of flumequine in the gut contents by Mg^{2+} and Ca^{2+} ions from sea water may have reduced the primary absorption and entero-hepatic cycling in seawater compared with freshwater conditions. Accordingly, compounds excreted in bile may have been more likely to be reabsorbed in fish held in fresh water than in sea water.

An alternative and not mutually exclusive mechanism for the more rapid elimination of flumequine under seawater holding conditions may have involved more rapid metabolism of flumequine. Flumequine elimination is due in part to glucuronidation, a process that has shown to be more rapid in sea water (Tachikawa & Sawamura 1994).

Different pharmacokinetic properties of quinolones in fresh water compared with sea water have previously been reported by Ishida (1992), who found that oxolinic acid was excreted more rapidly in seawater trout than in freshwater trout, and by Elston et al. (1994), who observed that difloxacin concentrations were higher in fish held in fresh water than in those adapted to seawater adapted fish.

High and persisting concentrations of flumequine in bone as demonstrated in the autoradiographic study may also be explained by the ability of the compound to make complexes with divalent cations like Ca^{2+} and Mg^{2+}. Furthermore, the high level of radioalabeled compound in the skin and the uveal tract of the eye may be explained by the ability of melanin to bind drugs, as documented by several studies (Lindquist & Ullberg 1972, Larsson 1993, Fukada & Sasaki 1994, Howells et al. 1994). Martinsen et al. (1994) found that residues of sarafloxacin, a quinolone closely related to flumequine, in the skin and the eye of cod persisted for a long time. Also, several studies on various fish species confirm that flumequine reaches high and persistent concentra-

tions in skin and bone (Steffenak et al. 1991, Elema et al. 1994, Malvisi et al. 1997, Plakas et al. 2000). Accordingly, the skin and bone tissues act as deep storage compartments that prolong the overall elimination of flumequine. Thus, the slow decrease in the blood concentration during the late elimination phase as observed in the present freshwater study may result from a release of flumequine from these deep reservoirs.

Flumequine distributed into the central nervous system (CNS) and high levels of the drug seemed to linger there for a long time, particularly after p.o. administration. CNS signs—rapid surface swimming and collision with the tank walls, in some cases associated with mortality—have been observed in several studies (O'Grady et al. 1988, Hansen & Horsberg 2000b) in flumequine-treated fish. Furthermore, CNS signs following flumequine administration have also been reported in mammalian species (Mevius et al. 1990).

In the present study we did not analyze for metabolites. Several studies (Schuppan et al. 1985, Mevius et al. 1990, Vree et al. 1994, Delmas et al. 1997) confirm that flumequine can be both oxidized and conjugated with glucuronic acid in mammals. In fish the metabolism of flumequine appears to be slow, and with the exception of bile only very low levels of metabolites have been detected (Van der Heijden et al. 1993, 1994, Samuelsen & Ervik 1997, Plakas et al. 2000), indicating that the total flumequine residues accounted for by means of the applied radioactive tracer techniques consist mainly of the parent compound.

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