Ivermectin Depuration in Atlantic Salmon (*Salmo salar*)

Myron Roth,*,† Gordon Rae,‡ Alister S. McGill,§ and Kenneth W. Young§

Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA Scotland, Scottish Salmon Growers Association, Drummond House, Scott Street, Perth, PH1 5EJ Scotland, and Ministry of Agriculture, Fisheries and Food, Torry Research Station, P.O. Box 31, Abbey Road, Aberdeen, AB9 8DG Scotland

The depuration of ivermectin (22,23-dihydroavermectin B_{1a}/B_{1b}) from Atlantic salmon (*Salmo salar*) muscle and skin was studied following nine weekly oral treatments, at a dose rate of 50.0 μg/kg of body weight per day. Depuration from both tissues followed first-order kinetics with slightly longer retention times being recorded for the skin ($t_{1/2}$ = 188.1 degree days (°D)) than for the muscle ($t_{1/2}$ = 120.4 °D). Residues of 7.2 ± 4.7 μg/kg could be detected in muscle tissues following 500 °D withdrawal, after which they fell below the limit of determination (2.0 μg/kg). In contrast, ivermectin residues could be detected in skin following 750 °D withdrawal (5.2 ± 1.5 μg/kg) but were not detected after 1000 °D. In light of these findings, the use of ivermectin as a potential chemotherapeutant for the control of sea lice infestations of cultured salmon is discussed.

INTRODUCTION

Infestations with parasitic copepods, *Lepeophtheirus salmonis* and several species from the genus *Caligus*, collectively referred to as salmon lice, represent one of the most serious disease problems currently facing the salmon farming industry. The control of sea lice is generally achieved through the use of the contact organophosphorus pesticides dichlorvos and trichlorfon (Brandal and Egidius, 1979; Rae, 1979; Reyes and Bravo, 1983; Grave et al., 1991). The method for treatment, which has been described in detail by Rae (1979) and Brandal and Egidius (1979), is very labor intensive and is not without several drawbacks which include difficulties in achieving the required dose rate (Wells et al., 1990), low therapeutic ratios (Salte et al., 1987; Horsberg et al., 1989), differential susceptibility of various life stages of the parasite (Wootten et al., 1982), and, most recently, reduced sensitivity, in susceptible life stages, in some populations of lice (Jones et al., 1992). Where large offshore oceanic cages are used, topical application of chemotherapeutants, as described by Rae (1979) and Brandal and Egidius (1979), is not practical. Given these difficulties, several alternative compounds and treatment methodologies have been investigated as alternatives for the control of sea lice (for review, see: Roth et al., 1993). In particular, ivermectin has been shown to be highly efficacious not only in the treatment of sea lice (Palmer et al., 1987; Smith et al., 1993) but also in the treatment of other species of parasitic copepods as well (Hyland and Adams, 1987; O'Halloran et al., 1992).

At present, there are no data available on the withdrawal of ivermectin from salmon flesh when used for sea lice control, which entails orally medicating fish at weekly intervals for several weeks (Smith et al., 1993). Høy et al. (1990) studied in detail the distribution of tritium-labeled ivermectin in Atlantic salmon following administration via an oral gavage. However, the study was limited to one treatment, had a limited depuration period, and did not distinguish between parent and metabolic byproducts. The present study was therefore initiated in order to obtain information on the depuration of ivermectin following a treatment regimen which more closely resembled that which would be used under practical conditions.

EXPERIMENTAL PROCEDURES

Forty-eight sea-acclimated salmon smolts were obtained from a salmon grower on the west coast of Scotland and transferred to a single polyethylene holding tank with a volume of 550 L and supplied with flow-through sea water at a rate of 4 L/min. Fish were acclimated for several weeks, during which they were offered pelleted salmon feed *ad libitum*.

Fish were given medicated feed after the method of Smith et al. (1993) with the following modifications. Throughout the trial, fish were fed at a rate of 1.5% body weight/day (based on the weights of 21 fish prior to the beginning of the experiment). Ivermectin (22,23-dihydroavermectin B_{1a}/B_{1b}) was obtained as a 1.0% ai w/w injectable solution (IVOMEC, Merck Sharpe & Dohme) and incorporated into the feed by mixing the appropriate amount of the drug into a precooled (35 °C) 5.0% gelatin solution which was then coated onto individual preweighed feed lots (1 mL of gelatin solution/12 g of feed). Ivermectin was incorporated into the feed to achieve a rate of 50.0 μg/kg of body weight per day once a week for a total of 9 weeks. Since the degradation half-life of ivermectin when stored in the dark at room temperature is 34 weeks (Halley et al., 1989), all medicated feed lots were prepared at the beginning of the experiment and stored in light-proof glass containers at 4 °C until used.

At the end of the medicated feeding period, fish were fed with unmedicated pellets, *ad libitum*, until the end of the sampling period. During the medicated feeding period, temperatures (measured on a daily basis) ranged from 8 to 10.5 °C (November to January). However, during the withdrawal period (January to May), temperatures became somewhat colder, reaching 6.0 °C (mid to late February) and then increasing to 9.5 °C by the end of the sampling period (midMay). Salinity (measured on a daily basis) did not fluctuate appreciably and ranged from 30‰ to 35‰ during the experimental period.

Given that metabolic activity in fish is directly related to temperature and the variability in temperature encountered over the course of the experimental period, depuration was measured in relation to degree days (°D). Degree days are defined here as the cumulative daily water temperature for each sample day. Thus, 3 days at a water temperature of 10 °C would equal 30 °D. Five fish were randomly selected from the group for residues 8.5 (24 h following the last feeding), 246, 500, 700, and 1000 °D from the last medicated feeding. Fish were killed with a blow to the head, weighed (wet), and then stored at −20 °C.

For residue analysis, samples were assayed by HPLC after the method of Norlander and Johnson (1990). Individual fish were

* Address correspondence to this author at Pacific National Group Ltd., Site 306, C13, Great Central Lake Rd., R.R. no. 3, Port Alberni, BC, V9Y 7L7 Canada.
† University of Stirling.
‡ Scottish Salmon Growers Association.
§ Torry Research Station.

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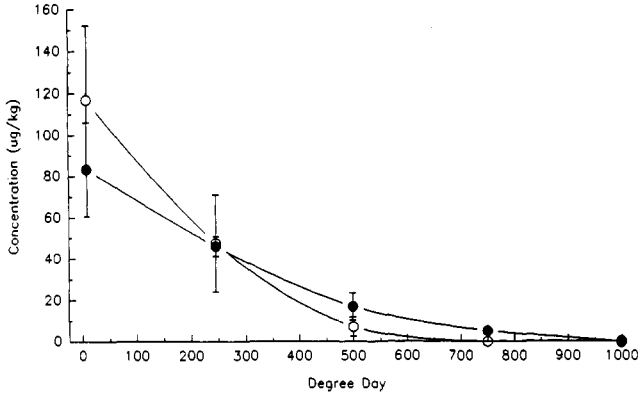

Figure 1. Muscle and skin concentrations of ivermectin in Atlantic salmon following nine weekly oral doses at 0.05 μg/kg (bars = SD): (●) skin and (○) muscle.

filleted and skinned, and the muscle/skin tissue was minced. Ivermectin residues were extracted from tissues into organic solvent and separated from coextractives using a C_8 solid-phase extraction cartridge. The eluate was concentrated, derivatized, and purified using a silica solid-phase extraction prior to quantitation by HPLC and fluorescence detection. HPLC was performed using a Hypersil 15 ODS column (5 μm, 250 - × 4.6-mm i.d.) and methanol/water (97/3 v/v) as the mobile phase. The derivatized ivermectin was quantified using fluorescence detection (Applied Biosystems, Anachem, Luton, U.K.) with an excitation wavelength of 364 nm and an emission wavelength of 470 nm. Any positive samples were quantified using a calibration curve constructed from an ivermectin standard (a kind gift from Merck, Sharp & Dohme Ltd., Hertford, U.K.) such that the ivermectin content of the sample fell between the limits of the detection curve. Quality control was achieved by spiking blank fish samples with 10.0 μg/kg of standard ivermectin followed by extraction through the full method. Recovery was 84%, CV 11.5; limit of determination (the lowest concentration that can be quantified with accuracy) was 2.0 μg/kg. To calculate tissue half-lives ($t_{1/2}$), concentration values were plotted on a semilogarithmic scale and fitted to a linear regression model (Brander et al., 1982).

RESULTS AND DISCUSSION

All fish appeared healthy and showed good appetites throughout the trial as indicated by weights recorded during the study which ranged from 167.6 ± 40.1 g (98–251) (pretreatment) to 352.4 ± 59.7 g (283–425) (1000 °D). One fish mortality was recorded 1 day prior to the last feeding (week 8). The cause of death was not determined. Earlier studies have shown that ivermectin readily crosses the blood–brain barrier and enters central nervous tissues (Høy et al., 1990), which may explain the negative side effects (sluggishness) and the toxicity of the compound when administered to salmon as a single, or replicate, dose (200.0–400.0 μg/kg) (Palmer et al., 1987; O'Halloran et al., 1992). Our data support that of O'Halloran et al. (1992) and Smith et al. (1993), suggesting that when administered at lower dose rates over a protracted period, toxic effects are avoided.

Ivermectin residue depletion from skin and muscle tissues during the withdrawal period is summarized in Figure 1. The total amount of ivermectin given (per kg/fish) was estimated to be 450.0 μg/kg. One day (8.5 °D) from the last medicated feeding, the results showed that the muscle and skin tissues contained 116.8 and 83.3 μg/kg, respectively (26% and 19%, respectively, of the total administered amount), suggesting that the compound does not accumulate in fish muscle or skin.

By 246 °D, muscle and skin residues had decreased to 47.6 and 46.0 μg/kg, suggesting higher rates of metabolism in the muscle. Following a further 250 °D, muscle residue levels had reduced to 7.2 μg/kg (1.6% of the administered dose), whereas skin residues had decreased to 17.2 μg/kg (3.8% of the total administered dose). No residues (parent compound) were detected in the muscle 750 or 1000 °D from the final medicated feeding. The skin was found to retain the compound somewhat longer than the muscle with residues of 5.2 μg/kg being detected after 750 °D. No residues were detected in the skin after 1000 °D. Depuration appeared to follow first-order kinetics with tissue half-life estimates of 120.4 °D (r = 0.9833) and 188.1 °D (r = 0.9915) for the muscle and skin, respectively.

The results are in general agreement with those of Høy et al. (1990) who reported that following a single application of tritium-labeled ivermectin via an oral gavage, 29% and 19% of the administered dose could be detected (in the form of parent compound and/or metabolites) in the liver, kidney, muscle, and blood 28.4 and 198.8 °D, respectively, from administration. However, Høy et al. (1990) also found that concentrations of ivermectin were highest in lipid-containing visceral organs (e.g., liver) which may represent tissues with potentially longer retention times than those reported in the present study. In contrast, Wislocki et al. (1989) reported that the structurally similar compound avermectin B_{1a} did not accumulate in bluegill sunfish (*Lepomis macrochirus*) following exposure via water, which was believed to be due to poor lipid solubility due to the relatively large size of the molecule.

The main route of excretion of ivermectin in fishes is via bile (Høy et al., 1990). From their study, Høy et al. (1990) reported that after 7.1 (1 day), 21.3, and 106.5 °D, the amount of parent compound excreted in the bile, as a percentage of the sample, was 77%, 44% and 42%, respectively. These data (Høy et al., 1990; present study) suggest that the depuration of ivermectin from fish tissues follows a biphasic process, similar to that observed with cattle and sheep (Fink and Porras, 1989). However, further work will be required to characterize the initial uptake and the subsequent depletion of ivermectin in fish following repeated (oral) applications.

With respect to the use of ivermectin as an oral chemotherapeutant for the control of sea lice, the present finding raises two important points. First, given the strong binding characteristics and stability of ivermectin in soil (Halley et al., 1989) and the insufficient data on lipid solubility, more data will be needed on the fate of the relatively high proportion of ivermectin which passes through the gut unabsorbed (Høy et al., 1990), lipid solubility, and the effect of fat content on depletion. Second, these results show that, at the dose rates/regimens used in the present study, salmon would require a minimum withdrawal period of 1000 °D to eliminate ivermectin residues (parent compound) from edible tissues, until maximum residue limits are set by regulatory agencies. Consequently, long withdrawal periods of 67 (at 15 °C) to 200 days (at 5 °C), depending on local temperature conditions, would be necessary following treatment, during which time fish may become reinfected and significant parasite damage may occur. In such circumstances, an alternative chemotherapeutant with short tissue residency times would be required for short-term lice control.

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