Aquaculture 321 (2011) 207–215

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effect of emamectin benzoate on transcriptional expression of cytochromes P450 and the multidrug transporters (Pgp and MRP1) in rainbow trout (*Oncorhynchus mykiss*) and the sea lice *Caligus rogercresseyi*

Juan Guillermo Cárcamo [a,*], Marcelo N. Aguilar [a], Claudia A. Barrientos [a], Constanza F. Carreño [a], Claudia A. Quezada [a], Carlos Bustos [b], René A. Manríquez [a], Rubén Avendaño-Herrera [c], Alejandro J. Yañez [a]

[a] Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
[b] Instituto de Ciencias Químicas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
[c] Laboratorio de Microbiología y Biología Molecular, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Viña del Mar, Chile

ARTICLE INFO

Article history:
Received 18 August 2011
Accepted 6 September 2011
Available online 17 September 2011

Keywords:
Cytochrome P450
Emamectin benzoate
Caligus rogercresseyi
Multidrug resistance proteins

ABSTRACT

Caligus rogercresseyi is a sea louse that affects salmon farming industry throughout the Southern hemisphere. In Chile, oral delivery of emamectin benzoate (EMB) to salmon was the main treatment used; however, *C. rogercresseyi* became resistant to EMB. The aims of this study were determine the effect of EMB on proteins involved in metabolism and drug resistance in rainbow trout (*Oncorhynchus mykiss*) and *C. rogercresseyi*. Since no commercial antibodies are available for these salmon's proteins, the expression of trout cytochrome P450 CYP2K1, CYP2M1, CYP3A and CYP3A27, and the multidrug transporters Pgp and MRP1 were investigated by semi-quantitative duplex RT-PCR. Trout specimens infested with *C. rogercresseyi* were medicated with EMB by a standard seven-day oral treatment in salmon farms from southern Chile, and collected between 10 and 25 days after the completion of medication. The samples examined were liver, muscle, gill, middle kidney and intestine from control and EMB treated rainbow trout, and *C. rogercresseyi's* total homogenates. In trout tissues most CYP and MDR gene expressions were up-regulated by EMB treatment, being MRP1 mRNA in all tissues, but particularly in the kidney and intestine, and CYP2M1 mRNA from muscle and gill, the most augmented. CYP3A, CYP3A27, CYP2K1 and Pgp mRNAs were slightly affected in the most tissues analyzed. In *C. rogercresseyi* minimal up-regulation of the expression for most of mRNA analyzed was observed, except for CYP2M1 mRNA, decreasing its expression by half, and CYP3A27 doubling its expression levels. Nonetheless, CYP3A mRNA expression was not detected in this parasite. These results suggest that treatment with EMB in salmon regulates the transcriptional expression of proteins involved in metabolism, distribution and elimination of endobiotics and xenobiotics, such as hormones and drugs, and even could affect the pharmacokinetics of EMB in the same treatment.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Sea lice, *Caligus rogercresseyi*, a copepods ectoparasite, severely affects the Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) farming industry in Southern Chile (Boxshall and Bravo, 2000), reducing the health status of fish and producing both direct and indirect economic losses. Since the first reports of the sea lice in 1982, a wide variety of antiparasitic agents has been used to control this parasite (Bravo et al., 2008), although since 2000 emamectin benzoate (EMB) was the only medicinal product allowed by the Chilean official authority to control *C. rogercresseyi*, now deltamethrin and diflubenzuron also became available.

Emamectin benzoate [(4″R)-4″-deoxy-4″-(methylamino) avermectin B1 benzoate] is a macrocyclic lactone semi-synthetic derivative of the avermectins developed for the control of insect pests (Mushtaq et al., 1996). In salmonids, oral EMB administration to treat *C. rogercresseyi* infestations is achieved through its inclusion in fish food premix (Grant, 2002), the parasite ingests the drug while feeding on salmon mucus and probably blood. However, the use of large amounts of this compound and the effect of a repetitive therapy with the same antiparasitic agent lead to the loss of sensitivity of *C. rogercresseyi* to EMB (Bravo et al., 2008).

To exert its neurotoxic actions, EMB stimulates the release of gamma-aminobutyric acid (GABA) from nerve endings and therefore it enhances the binding of GABA to receptor sites on the post-junction membrane of muscle cells, leading to hyperpolarization and the consequent inhibition of neurotransmission (Grant, 2002). In this context, EMB must cross several cell barriers from intake, absorption and distribution, in order to reach an adequate concentration in salmon blood

* Corresponding author. Tel.: +56 63 293413; fax: +56 63 221466.
E-mail address: gcarcamo@uach.cl (J.G. Cárcamo).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.09.012

and mucus, and then be ingested by *C. rogercresseyi*, pass through other cellular barriers to reach their molecular targets at the parasite's peripheral nervous system (Davies, 2000; Sevatdal et al., 2005).

Metabolic drug inactivation processes and xenobiotic biotransformation in metazoans are associated to the activity and expression levels of phase I, II, and III families of xenobiotic metabolizing enzymes/proteins. This system consists of an array of enzymes/proteins that metabolize xenobiotics and endobiotics via various reactions and eventually export these metabolites from the cell (Nakata et al., 2006; Xu et al., 2005). Phase I, represented mainly by cytochrome P450 (CYP) multi enzymatic complex, includes mostly hydrolysis, oxidation, and reduction reactions, by adding or exposing functional groups (e.g., hydroxyl, sulfhydryl, carboxyl, and amino) (Danielson, 2002). Phase II includes conjugation or synthetic reactions (e.g., methylation, glucuronidation, and sulfation) to further increase the solubility of the compound (Kleinow et al., 1987; Livingstone, 1998). Some highly expressed CYP metabolizing enzymes have already been described in rainbow trout liver, gills and olfactory rosettes as well as in other fish species, including different CYP forms such as CYP1A, CYP2K1, CYP2M1, CYP3A and CYP3A27 (Matsuo et al., 2008; Meucci and Arukwe, 2006; Thibaut et al., 2002). Phase III proteins, are constituted by membrane-residing transporters, which export metabolites, conjugated or not, from the cell to the outside. Among phase III proteins, the multidrug resistance transporters (MDR proteins) such as P-glycoprotein (Pgp) and multidrug resistance-associated proteins (MRPs) (Cascorbi, 2006; Schinkel and Jonker, 2003) are among the most studied. Pgp and MRP1 have been linked to macrocyclic lactones resistance such as avermectins, ivermectins and moxidectins (Kiki-Mvouaka et al., 2010; Lespine et al., 2008; Lespine et al., 2006). Moreover, ivermectin has been described as a substrate of CYP3A sub-family member (Zeng et al., 1998), and avermectins as substrate for Pgp (Ayrton and Morgan, 2001; Lespine et al., 2008) and MRP1 (Brayden and Griffin, 2008). Some Pgp substrates and inhibitors can also be substrates for CYP enzymes; in this context, CYP3A is one of the subfamilies co-expressed with Pgp in the liver and intestine with overlapping substrate specificity (Brayden and Griffin, 2008), which probably act synergistically to reduce oral absorption in prone individuals to certain xenobiotics (Benet et al., 2004).

From this point of view, the identification and characterization of MDR proteins and CYP enzymes involved in absorptive processes and bioavailability of EMB, and the observation whether these proteins are transcriptionally affected by EMB, could highlight the mechanisms of xenobiotic metabolism and elimination involved in EMB resistance in different tissues of rainbow trout and in sea louse, and about the pharmacokinetic effect of this antiparasitic drug over pharmacological treatments performed during at least the month after completion of medication with EMB in salmon.

The current investigation was aimed to determinate by semi-quantitative RT-PCR whether EMB modulates the transcriptional expression of CYP metabolizing enzymes (CYP2K1, CYP2M1, CYP3A and CYP3A27) and MDR transporters (Pgp and MRP1) in *C. rogercresseyi* and in different tissues of its host, rainbow trout.

2. Methods

2.1. Samples

Adult rainbow trout (1.2 to 2.2 kg) affected by *C. rogercresseyi* were obtained from different salmon farms belonging to three large aquaculture companies in Chile located in the Southern Chile (Trusal, Multiexport Foods and Providencia Fish Farms). Three groups of infected fish (one group per farms) had been previously treated with EMB by oral administering 50 μg per kilogram fish per day for seven days. Another three groups with fish without the addition of the EMB, but run simultaneously in exactly the same conditions as described above were included as controls. Five specimens with and without EMB treatments were collected between 10 and 25 days after completion of medication. Sampled fish were killed by an overdose of anesthetic (BZ-20®) in a saltwater solution and immediately subjected to a full necropsy. Tissue samples and adult *C. rogercresseyi* were aseptically collected from each fish for RNA extraction. For this, portions of organs (muscle, gill, liver, intestine and middle kidney) from each fish were stored at −20 °C in RNAsafer™ solution (RNA Stabilizer Reagent, Omega Bio-Tek Inc.) until to be used for RNA extraction. At the same time, adult *C. rogercresseyi* were collected with forceps from anaesthetized control and EMB treated fish and stored in RNAsafer™ solution.

2.2. RNA isolation

Total RNA was extracted from 150 mg of each rainbow trout tissues as well as from 5 to 6 specimens of *C. rogercresseyi* using the Chomczynski/phenol method according to Chomczynski and Sacchi (2006). The concentration and quality of each RNA sample were examined at 260 nm, diluted with ultra-pure water when was necessary to adjust to a concentration of 1.5–2.0 μg μl^{-1} and stored at −80 °C.

2.3. Synthesis of cDNA and polymerase chain reaction (PCR)

The synthesis of cDNA was performed using the commercial kit SuperScript™ II RT (Invitrogen), which included all the reagents needed for the synthesized first-strand cDNA, with the exception of the RNA (1.5–2.0 μg). After the first step was finished, 1 μl cDNA was used as template for the second step. All PCR amplifications were performed with the commercial kit GoTaq® Green Master Mix (Promega) according to manufacturer's protocol.

The duplex PCR protocol started with denaturation (94 °C for 3 min) followed by 35 cycles at 94 °C for 30 s (denaturation), 55–64 °C for 30 s (annealing) and 72 °C for 30 s (DNA chain extension) with a final extension at 72 °C for 10 min. The reactions were performed in a Swift Maxi Gradient Thermal Cycler (Esco Micro Pte Ltd). The duplex PCR annealing temperatures tested ranged from 55 to 64 °C due to the type of samples (i.e. fish organs or *C. rogercresseyi* pool) and primer pairs used. To amplify CYP metabolizing enzymes we used 4 primer pairs (Matsuo et al., 2008). The partial cds sequence of *O. mykiss* Pgp (P-glycoprotein (Abcb1) mRNA, GenBank: AY863423), the human MRP1 mRNA sequence (NCBI accession number: NM 019898.2), the *O. mykiss* MRP1 mRNA sequence (NCBI accession number: NM 001168330), and the *S. salar* β-actin mRNA sequence (NCBI accession number: BT047241) were retrieved from the GenBank database and analyzed employing the *AmplifX 1.5* software to design pairs of primers for Pgp and β-actin mRNAs, and two pairs for the MRP1 mRNA. The sequences of the all primer pairs for PCR and the expected size of PCR products with these primers are shown in Table 1.

Duplex PCR reactions (β-actin primers plus CYPs or MDRs primers) were carried out in triplicate using the cDNA obtained from 3 independent RNA extractions per each sample.

2.4. Analysis of PCR products

Amplified products were detected by horizontal 2% (w/v) agarose gel electrophoresis for 60 min at 100 V in TAE 1× (0.04 M Tris, 0.0001 M EDTA, pH 8.0) electrophoresis buffer, visualized using SYBR® Safe DNA gel stain (Invitrogen), photographed under UV light and computer digitized using Syngene Bio Imaging System and Gene Snap Software. A 100 to 3000 bp ladder (Winkler) was used as a molecular mass marker. The presence of a single product of the appropriate size, identical to the reference experiments, was considered as a positive result.

To quantify, β-actin was used as housekeeping gene (load control), the ratio obtained between the intensities (pixels) of amplification bands of the mRNA under study (CYP or MDR) and β-actin was calculated for both the control and EMB treated samples. The values are shown as mean ± standard deviations (SD) and correspond to

Download English Version:

https://daneshyari.com/en/article/2422845

Download Persian Version:

https://daneshyari.com/article/2422845

Daneshyari.com