Toxicon 51 (2008) 1431–1439

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Proteomic and phosphoproteomic analysis of cellular responses in medaka fish (*Oryzias latipes*) following oral gavage with microcystin-LR ☆

K. Mezhoud [a], A.L. Bauchet [b], S. Château-Joubert [b], D. Praseuth [c], A. Marie [d], J.C. François [c], J.J. Fontaine [b], J.P. Jaeg [e], J.P. Cravedi [e], S. Puiseux-Dao [a], M. Edery [a,*]

[a] USM 505/EA 4105, Département de Régulations, Développement et Diversité Moléculaire, Ecosystèmes et Interactions Toxiques, Muséum National d'Histoire Naturelle, 12 Rue Buffon, F-75231 Paris Cedex 05, France

[b] Département de Pathologie, Ecole Nationale Vétérinaire d' Alfort, 7 Avenue du Général de Gaulle, F-94704 Maisons-Alfort Cedex, France

[c] CNRS, UMR 5153; INSERM, U565; USM 503, Département de Régulations, Développement et Diversité Moléculaire, Régulations et Dynamique des Génomes, Muséum National d'Histoire Naturelle, 57 Rue Cuvier, F-75231 Paris Cedex 05, France

[d] Plateforme de Spectrométrie de Masse et de Protéomique, CNRS, UMR 5154, Département de Régulations, Développement et Diversité Moléculaire, Chimie et Biochimie des Substances Naturelles, Muséum National d'Histoire Naturelle, 63 Rue Buffon, F-75231 Paris Cedex 05, France

[e] UMR 1089 Xénobiotiques, INRA Toulouse. 180 Chemin de Tournefeuille, BP3, F-31931 Toulouse Cedex 9, France

ARTICLE INFO

Article history:
Received 22 January 2008
Received in revised form
17 March 2008
Accepted 18 March 2008
Available online 25 March 2008

Keywords:
Microcystin-LR-medaka-proteomics
Phosphorylation
Anatomo-pathology

ABSTRACT

Chronic and subchronic toxicity resulting from exposure to microcystins (MCs) receives increasing attention due to the risk of bioaccumulation of these toxins by aquatic animals, including fish. The mechanisms of action of MCs that target the liver, involve modifications of protein phosphorylation resulting from phosphatases 1 and 2A inhibition. Therefore, studying phosphoprotein modifications by using a specific phosphoprotein stain Pro-Q Diamond in fish liver contaminated with MC-leucine–arginine (MC-LR), the most toxic MC, should help dissecting disturbed signaling and metabolic networks. We have recently used this technology to identify several proteins that are modulated either in expression or phosphorylation in the liver of medaka following short-term exposure to MC-LR by balneation. In the present study, we have decided to use an alternative way of introducing the toxin into fish; that is by gavage (force-feeding). This was first achieved using tritiated MC-LR and allowed us to quantify the quantity of toxin incorporated into fish and to demonstrate that the toxin is mainly accumulated in liver. Afterwards a proteomics study limited to liver cytosolic proteins of contaminated animals showed that several proteins were up or down regulated either in quantity or in phosphorylation or both. Some of them had been previously detected as modified in balneation experiments but new molecules were identified as involved in signal transduction pathways activated by the toxin. In addition, in the conditions used

Abbreviations: MCs, microcystins; MC-LR, microcystin-LR; ROS, reactive oxygen species; MPT, mitochondrial permeability transition; 2-D, two-dimensional; 2-DE, two-dimensional electrophoresis; PAGE, polyacrylamide gel electrophoresis; EST, expressed sequence tag; IDA, information-dependent acquisition; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; TRAIL, TNF-related apoptosis-inducing ligand; TNF, tumor necrosis factor; NCBI, National Center of Biological Information; NCBInr, non-redundant protein database; NCBI EST, NCBI expressed sequence tag database; Blast, basic local alignment search tool; Blastp, Blast for protein database; rpsblast, reverse position specific Blast (search conserved domains on a protein); tblastn, translate Blast nucleotide; i.d., internal diameter.

☆ *Ethical statement*: The animals were handled in accordance with European Union regulations concerning the protection of experimental animals.

* Corresponding author. Tel.: +33 1 40 79 32 12; fax: +33 1 40 79 35 94.
E-mail address: medery@mnhn.fr (M. Edery).

0041-0101/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2008.03.017

(5 μg toxin/g body weight) anatomopathological studies supported a process of apoptonecrosis established after 24 h, which was suggested to proceed by the evolution of some of the proteins after 2 h contamination.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The toxic potency of bloom-forming cyanobacteria in eutrophic surface waters has caused increasing concern over the last decade. Rapid advances have been made in the study of chemically diverse cyanotoxins produced by these cyanobacteria, especially microcystins (MCs) (Cohen, 1989).

MCs, mainly MC-leucine–arginine (MC-LR), which is the most frequently observed, are known for a long time as hepatotoxins since natural intoxications are associated with hepatic lesions. Experimental intoxications in mammals (Dawson, 1998; Hooser, 2000; Guzman and Solter, 2002) and fishes (Tencalla et al., 1994; Williams et al., 1995; Fischer and Dietrich, 2000) showed liver lesions: hemorrhages, necrosis, cellular hypertrophy, glycogen depletion. Occurrence of apoptotic cells and dissociation of liver sheets have been described. Such effects are considered as resulting from protein phosphatase PP1 and PP2A inhibition (Chen et al., 2004). These toxins are able to penetrate liver cell membranes through a bile acid carrier and induce changes such as overphosphorylation of liver enzymes, liver necrosis, and even deadly intrahepatic bleeding (Falconer and Yeung, 1992). There is evidence that these adverse effects are closely related to oxidative stress processes and free radicals production (Hermansky et al., 1990; Ding et al., 1998). These cyanotoxins can be harmful to fish in natural conditions and aquaculture when MCs producing cyanobacteria are blooming. Fish behavior and productivity should be affected.

Zebra fish and medaka are excellent models to study toxic disturbances produced by pollutants and natural toxins (Hinton et al., 2005). Thus, we performed experiments on MC-LR effects on medaka. In a recent study, we transferred fishes into contaminated water to analyze effects in balneation conditions, which are the most natural and we exposed the fishes to the toxin in short experiments (1–2 h maximum) (Mezhoud et al., 2008). In order to introduce an alternative way of exposing fishes to toxin and to introduce a more efficient way of quantifying the uptake of toxin by fish, we decided to intoxicate fishes by force-feeding, which also allows to analyze the long-term effects of the toxin. This study is the second in a series of publications, which study the effects of MC-LR exposure in Japanese ricefish. Whereas the first study (Mezhoud et al., 2008) focuses on exposure through water, the current study investigates exposure through food intake. Since aquatic animals as fishes may be contaminated in their natural environment either through contaminated water or by ingesting contaminated food, assessing the differences in effects resulting from different exposure routes certainly is an important issue. In addition, the present study presents a more global view of the effects of the toxin by analyzing the kinetics and the anatomopathological effects of exposure to MC, which are definitively requested steps to integrate the proteomics study.

The previous experiments using balneation has allowed us to show that in short experiments (1 h or less), alterations of the proteome and phosphoproteome could be detected (Mezhoud et al., 2008). The results indicated that early responses affected key metabolic pathways implied in cell proliferation and cell death.

The present paper on MC-LR force-fed medakas reports results concerning radioactive MC-LR distribution, liver histopathological changes and proteomic approaches used to collect data on proteome and phosphoproteome responses to the cyanotoxin.

2. Materials and methods

Biological material, sample preparation and polyacrylamide gel electrophoresis (PAGE), protein chemistry, sample preparation and ESI-qTOF mass spectrometry were previously described (Mezhoud et al., 2008).

2.1. Toxin radiolabeling, oral gavage with tritiated MC-LR and organ sampling

Radiolabeling of the toxin was performed according to a technique developed by A. Humpage (unpublished data): the unusual amino acid *N*-methyl dehydroalanine (MDha) at position 7 was reduced by a sodium boro[3H]-hydride solution.

Adult medaka fishes (16 with 1 g weight) were force-fed with 10 μl of the tritiated MC-LR solution thanks to a 0.9 mm diameter cannula combined to a 50 μl Hamilton syringe. Fishes were collected at different time intervals: 5 after 3 h, 5 after 24 h, and 6 after 72 h. Fishes were anesthetized in ice-cold water, sacrificed and dissected under a Leica magnifying glass. Various organs were dissected from fishes: liver with gall bladder, abdominal digestive tract with spleen, gonads, kidneys, tail and the rest of the carcass. Each piece was weighed and placed in a paper cup.

Samples were oxidized by complete combustion with an Oxidizer Packard 307. Water gathered by the combustion was placed in a 10 ml plastic vial and mixed with liquid scintillation cocktails (14 ml Monophase S Perkin Elmer and 5 ml Permafluor Perkin Elmer). Tank water samples (1 ml) were performed at 3, 18, 24, 43, 51, 66 and 72 h after gavage; each sample was mixed with 8 ml liquid scintillation cocktail (Ultima gold) in a plastic vial. Radioactivity present in each plastic vial was analyzed with a liquid scintillation counter (1500 Tricarb Packard). Values obtained for each sample were expressed in

Download English Version:

https://daneshyari.com/en/article/2066629

Download Persian Version:

https://daneshyari.com/article/2066629

Daneshyari.com