Toxicon 124 (2016) 15–25

Contents lists available at ScienceDirect

Toxicon

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

Effects of exposure to microcystin-LR at environmentally relevant concentrations on the metabolism of thyroid hormones in adult zebrafish (*Danio rerio*)

Zidong Liu [a, b, c], Dapeng Li [a, b, c, *], Qing Hu [a, b, c], Rong Tang [a, b, c], Li Li [a, b, c]

[a] College of Fisheries, Huazhong Agricultural University, Wuhan, 430070, PR China
[b] Hubei Provincial Engineering Laboratory for Pond Aquaculture, Wuhan, 430070, PR China
[c] Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, Wuhan, 430070, PR China

ARTICLE INFO

Article history:
Received 14 August 2016
Received in revised form
31 October 2016
Accepted 3 November 2016
Available online 4 November 2016

Keywords:
Microcystin-LR
Thyroid hormones
Histology
Iodothyronine deiodinases
Zebrafish

ABSTRACT

Microcystin-LR (MC-LR) has the potential to disturb thyroid hormone homeostasis. However, the effects of MC-LR at environmentally relevant concentrations on the thyroid system in adult fish are still unclear. In this study, adult zebrafish were exposed to 0, 1, 5, and 25 μg/L MC-LR for 7, 14, 21, and 28 days. Whole-body thyroid hormones (THs) levels and thyroid follicle histology were used to assess thyroid function. The transcription of corticotropin-releasing hormone (*crh*), thyroid-stimulating hormone (*tsh*), transthyretin (*ttr*), thyroid hormone receptors (*trs*) genes, and the activities of iodothyronine deiodinases (IDs) were investigated to study the process of TH metabolism disruption. No differences in the histopathology of thyroid follicles and unchanged T_4 levels were observed in adult zebrafish. A significant decline in T_3 levels associated with a decrease in ID2 activity in male zebrafish was observed at 21 days exposure. Moreover, the mRNA expression of *tsh*, *ttr* and *trs* appeared to be a dynamic process as expression first decreased and then increased with continued exposure. These results indicated that exposure to MC-LR did not inhibit the production of TH. The decrease in ID2 activity may be an important factor in the decline of T_3 levels. Furthermore, it seems that the fish triggered a compensatory mechanism to maintain TH homeostasis in respond to environmental concentrations of MC-LR which induced TH disruption.

1. Introduction

Thyroid hormones (THs) play pivotal roles in the regulation of fundamental and basic physiological processes (Geven et al., 2009), such as growth, development, and reproduction (Johnson and Lema, 2011; Swapna et al., 2006). In most teleosts, the thyroid tissue is scattered diffusely in the pharyngeal region without forming a compact gland (Heijlen et al., 2013; Wendl et al., 2002). As in other vertebrates, the thyroid tissue consists of follicles formed by a single layer of epithelial cells surrounding an extracellular lumen containing a proteinaceous colloid (Brown et al., 2004). In fish, the main TH secreted by the thyroid gland is thyroxine (T_4), while the most active form of TH is 3, 5, 3′-triiodothyronine (T_3) (Eales and Brown, 1993). T_3 is generated almost entirely in the peripheral tissues by deiodination of T_4 (Heijlen et al., 2013). Deiodination regulates plasma and tissue TH levels and the biological activity of TH, and the iodothyronine deiodinases (IDs) are exclusive enzymes responsible for TH deiodination (Germain and Galton, 1997).

Similar to mammals, teleosts also express three types of IDs termed type I(ID1), type II(ID2), and type III (ID3) (Orozco and Valverde, 2005). Each ID has a different role in regulating TH metabolism, and catalyze the removal of iodine atoms from either the outer-ring or inner-ring of THs. Different catalysis methods can generate more active or less active forms of TH (Köhrle, 1999, 2000). ID1 is a dual-function deiodinase because it is capable of catalyzing both activation and inactivation of THs (Heijlen et al., 2013). In fish, however, both ID1 and ID2 act as an outer-ring ID to convert T_4 to the more active form T_3, while ID3 acts as an inner-ring ID to convert T_4 or T_3 to inactive forms including reverse triiodothyronine (rT_3) and diiodothyronine (T_2) (Orozco and Valverde, 2005; St. Germain et al., 2009). The activities of IDs play a key role in TH metabolism. However, these enzymes are

* Corresponding author. College of Fisheries, Huazhong Agricultural University, 1 Shizishan Street, Wuhan, 430070, PR China.
E-mail address: ldp@mail.hzau.edu.cn (D. Li).

http://dx.doi.org/10.1016/j.toxicon.2016.11.004
0041-0101/

very sensitive and are susceptible to environmental contaminants (Picard-Aitken et al., 2007).

Toxin-producing harmful algal blooms occur worldwide and are a major ecological concern. Cyanobacterial blooms are especially important as they can produce many types of microcystins (MCs), which are natural environmental contaminants. Microcystin-LR (MC-LR) is generally recognized as being the most toxic (Codd et al., 2005; Luckas et al., 2005), and concentrations in waters often exceed the World Health Organization advisory level of 1 μg/L (Chorus et al., 2000). One study reported that MC concentrations can even reach 57–78 μg/L (Zimba et al., 2001). However, the concentrations of MCs during most cyanobacterial blooms in lakes usually range from 0.1 to 10 μg/L (Jiang et al., 2011). In the environment, fish usually encounter MCs through the ingestion of whole cells or passively by contact with surrounding water, or a combination of both (Malbrouck and Kestemont, 2006). The mechanisms of MC toxicity in fish are similar to those reported in mammals. The liver is the major target organ and the primary mechanism of MC toxicity is inhibition of protein phosphatases 1 and 2A (Fischer et al., 2000; Fujiki and Suganuma, 1993).

The majority of studies have focused on reproduction, development, and bioaccumulation of MC toxicity in fish (Best et al., 2002; Fischer and Dietrich, 2000; Setlikova and Wiegand, 2009; Zhang et al., 2007). Recently, the effects of MCs on thyroid endocrine disruption have attracted growing interest. Studies have reported that MCs can significantly reduce TH levels in crucian carp (*Carassius auratus*) (Li et al., 2008), and alter the expression of genes involved in the hypothalamic-pituitary-thyroid (HPT) axis in zebrafish larvae (Yan et al., 2012). However, there are few studies on the effects of subacute exposure to MC-LR at environmentally relevant concentrations. In addition, the majority of studies on MCs or MC-LR has focused on the early life stages of fish, and much less is known about aqueous exposure to MC in adult fish, especially the effects of MC-LR on TH disruption in adult fish. Although larval fish are generally more sensitive to environmental contaminants than adults (Li et al., 2011), a complete assessment of the potential disruption caused by MC-LR on the thyroid system in adult zebrafish is also very meaningful. Abnormal levels of TH usually cause adverse effects on reproduction (Blanton and Specker, 2007; Cyr et al., 1998; Parhar et al., 2000). Moreover, environmental contamination usually has a different influence on male and female fish (Johnson and Lema, 2011; Liu et al., 2010). The sex-specific difference can provide a comprehensive understanding of the disruptive mechanism of chemicals. To date, it is not clear whether MC-LR disrupts TH in adult fish. Considering low concentrations (less than 2 μg/L) of MC-LR can significantly interrupt the various metabolic processes in zebrafish liver (Wang et al., 2010), we hypothesized that MC-LR probably disrupts the transcription of selected genes involved in TH metabolism and the activities of IDs in liver, which in turn influence the TH level and disturb normal physiological function in fish.

The objective of the present study was to investigate the effects of subacute exposure to environmentally relevant concentrations of MC-LR on thyroid endocrine disruption in adult zebrafish. The concentrations of T_3 and T_4, the levels of physiologically relevant free T_3 (FT_3) and free T_4 (FT_4), as well as thyroid follicle histology were used as direct endpoints to assess thyroid disruption. Furthermore, the transcription of transthyretin (*ttr*) and thyroid hormone receptors (*trs*) genes, and the activity of IDs were also determined in adult zebrafish. The assessment of these factors involved in TH synthesis and metabolism allows for a more complete understanding of the mechanism of thyroid disruption in adult zebrafish exposed to MC-LR at environmentally relevant concentrations.

2. Materials and methods

2.1. Chemicals and fish

Microcystin-LR (MC-LR, purity ≥ 95%) was purchased from Enzo Life Sciences (Lausen, Switzerland). Healthy three months old zebrafish (*Danio rerio*) of both sexes used in this study originated from the Institute of Hydrobiology, Chinese Academy of Sciences, China. All other chemicals used in this study were analytical grade.

2.2. Experimental design

A stock solution of MC-LR was prepared by dissolving the toxin in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA). A range of different concentrations for exposure (0, 1, 5, and 25 μg/L) were prepared by diluting the stock solution with dechlorinated tap water. The final concentration of DMSO in aquarium water in the control and treatment groups was 0.001% (vol/vol).

Male and female fish were maintained in two different glass tanks respectively, containing dechlorinated tap water at a constant temperature (25 ± 1 °C) under a 14 h light/10 h dark photoperiod for 15 days in our laboratory prior to the experiments. During experimental exposure, the fish were randomly distributed into ninety-six glass tanks (12 fish/tank, forty-eight tanks for male fish and forty-eight tanks for female fish) and assigned to the following treatments: three tanks for each exposure concentration (0, 1, 5, and 25 μg/L MC-LR) and exposed for 7, 14, 21, and 28 days, respectively. One third of the exposure solution in each tank was renewed with fresh solution containing the appropriate concentration of MC-LR every day. A commercial ELISA kit for MC-LR detection, purchased from J & Q Environmental Technologies Co., Ltd., was used to monitor the MC-LR concentration in the solutions. The MC-LR concentration in each tank was measured after 0, 3, 6, 9, 14, 21, and 28 days exposure, respectively. The results indicated that there were no significant differences between the target doses and measured concentrations of MC-LR in the tanks during the experimental period (Table 1). The control group received 0.001% (vol/vol) DMSO without MC-LR. Less than 10% mortality was observed in all the treatment groups during experimentation.

Fish from the control and treatment tanks were sampled on experimental days 0, 7, 14, 21, and 28. The sampled fish in each experimental group were anesthetized with tricaine methanesulfonate (MS222, Sigma), and the heads from three fish per tank were severed and fixed in Bouin's fixative for histological examination. Whole livers were dissected from three fish per tank and preserved at −80 °C for gene expression and IDs activity assays. The remainder of whole fish was also stored at −80 °C for measurement of thyroid hormones. All experiments were carried out with approval from the Institutional Animal Care and Use Committee (IACUC) of Huazhong Agricultural University (Wuhan, China), and were performed in accordance with the International Guiding Principles for Biomedical Research Involving Animals as promulgated by the Society for the Study of Reproduction.

Table 1
Measured concentrations of MC-LR in the solutions.

	MC-LR concentrations (μg/L)			
Target doses of MC-LR	control	1.0	5.0	25.0
Measured MC-LR in solutions	0.00	0.87 ± 0.07	4.25 ± 0.41	23.40 ± 0.83

Data are denoted as mean ± SD.