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Microcystin-LR induced thyroid dysfunction and metabolic disorders in mice

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ABSTRACT

There is growing evidence that microcystins (MCs) act as hazardous materials and can disrupt the endocrine systems of animals. However, the response of thyroid function and the related energy metabolism following MCs exposure is still unknown. In the present study, mice were injected intraperitoneally (i.p.) with doses of either 5 or 20 μg/kg MC-LR for 4 weeks. We report, for the first time, that mice exposed to 20 μg/kg MC-LR showed disrupted glucose, triglyceride and cholesterol metabolism with obvious symptoms of hyperphagia, polydipsia, and weight loss. The circulating thyroid hormone (TH) levels in mice following MC-LR exposure were detected. Significantly increased free triiodothyronine (FT_3) and decreased free thyroxin (FT_4) were largely responsible for the physiological aberrations and metabolic disorders observed in mice after the 20 μg/kg MC-LR exposure. Increased expression of TH receptor (Trα) and mTOR expression in the brain after the 20 μg/kg MC-LR exposure suggests that the increased FT_3 enhanced mTOR signaling subsequently led to hyperphagia and elevated energy expenditure in mice. Furthermore, several genes involved in glucose homeostasis and lipid metabolism, which have been identified affected by TH, were also differentially expressed after MC-LR exposure. The above results clearly showed that mice exposed to MC-LR experienced thyroid dysfunction and its downstream functional changes, and are useful to better understand the endocrine toxicity of MC-LR to mammals or even humans.

1. Introduction

The occurrence of toxic cyanobacterial blooms in freshwaters has generated worldwide concern. Microcystins (MCs) are secondary metabolites produced by certain species of cyanobacteria and can pose a threat to the environment. To date, more than 90 different structural analogues of MCs have been identified, while microcystin-LR (MC-LR) is the most toxic structural variant (Ufelmann et al., 2012). The World Health Organization WHO (1998) has recommended a limit of 1 μg/L for MC in drinking water. However, this threshold has been exceeded in a number of water bodies. High levels of MC (14.3–20.0 μg/L) in August near the Maumee River in the western basin of Lake Erie were obtained by Rinta-Kanto et al. (2005). Song et al. (2007) recorded dissolved MC concentrations of nearly 7 μg/L in Lake Taihu, the third largest lake in China. Chen et al. (2009) reported MC concentrations of 3.74 μg/L in July in Lake Chaohu with an estimated daily intake in the range of 2.2–3.9 μg MC by fishers. These investigations indicate a health risk for populations with high levels of MC exposure.

MCs are known mainly for their hepatotoxicity due to their inhibitory activity of phosphatases PP1 and PP2A (Falconer and Yeung, 1992). However, MCs also can accumulate in gonads, kidney, and brain, resulting in reproductive toxicity, nephrotoxicity and neurotoxicity (Ondracek et al., 2012; Papadimitriou et al., 2010; Wang et al., 2008). Recently, both laboratory and field studies indicated that MCs can act as an endocrine disruptor (ED) in animals (Rogers et al., 2011 Zhao et al., 2015). EDs are able to disrupt the activity of the endocrine system and consequently modulate the metabolic activity of target structures (Dorabawila and Gupta, 2005). However, most evidence originates from studies

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on the reproductive system; in contrast, the response of thyroid function and the related energy metabolism to MCs exposure is still largely unknown.

Recently, greater attention has been devoted to the effect of environmental chemicals on the synthesis of thyroid hormones (THs) (Boas et al., 2006). There are two principal thyroid hormones: thyroxin (T_4) and triiodothyronine (T_3). The biologically active form of TH is T_3, although T_4 is the most abundant TH in the blood (Song et al., 2011). In humans, more than 99% of THs are bound to the carrier protein, and only a very small fraction of the circulating hormone is in the free state. The free T_4 (FT_4) and free T_3 (FT_3), which can penetrate the cytoplasm and nucleus membrane and combine with the hormone receptors to trigger the unique gene, are the biologically active form of THs responsible for metabolic processes within animal body (Tahboub and Arafah, 2009; Lin et al., 2010). Hence, measurement of plasma free THs concentrations is essential for the diagnosis of thyroid dysfunction (Bartalena et al., 1996).

THs play an important role in thermogenesis, regulation of energy expenditure and maintenance of metabolic homeostasis (Silva, 2006; Cheng et al., 2010; Brent, 2012). Thyroid dysfunction could result in hyperthyroidism or hypothyroidism, leading to marked alterations in energy balance (Huang and Liaw, 1995). Hyperthyroidism is a clinical syndrome in which overactive cells within the thyroid gland produce large amounts of T_3 and T_4, with a resulting excess of circulating free THs and increased metabolic rate (López et al., 2013). Many diseases and conditions can cause hyperthyroidism, including but not limited to thyroiditis, noncancerous growths of the thyroid gland or pituitary gland, and taking too much TH or iodine. A symptom of hyperthyroidism is weight loss despite increased food intake, because energy intake fails to meet the increased caloric demands associated with increased energy expenditure (Silva, 2006). Hypothyroidism, by contrast, is associated with decreased metabolic rate. The syndrome of hypothyroidism in rodents is similar to that in humans (Bianco et al., 2005). THs can influence diverse metabolic pathways involved in food intake, energy expenditure, and lipid and glucose metabolism (López et al., 2013). There is growing evidence that exposure to environmental chemicals may be associated with the occurrence of hyperthyroidism or hypothyroidism, resulting in metabolic disorders in exposed animals; indeed, environmentally realistic doses may be affecting human thyroid homeostasis (Dye et al., 2007; Blake et al., 2011; Goldner et al., 2013; Winquist and Steenland, 2014).

In the present study, we provide evidence that MC-LR exposure disrupts thyroid function in mice, causing obvious metabolic disorders, such as hyperphagia, polydipsia, polyuria, and weight loss accompanied with impaired lipid and glucose metabolism. The molecular mechanisms involved in the regulation of THs in metabolic disorders were also explored. These results provide novel insights into the endocrine toxicity of MC-LR in animals and also are useful in further understanding its potential risk to human health.

2. Materials and methods

2.1. Animals and treatments

We used adult male Balb/c mice aged 6 weeks purchased from Wuhan Institute of Virology, CAS. Mice were assigned randomly to 3 groups (one control and two treatments) of 15 each and were housed in a laboratory animal center to acclimate to the laboratory environment for 1 week before treatment. Five mice were housed per cage, with three cages per group. All cages were located in animal room with temperature ranging from 20 to 22 °C with a 12:12 light-dark cycle, and given free access to standard rodent pellet diet and water. All procedures carried out on animals were approved by the Institutional Animal Care and Use Committee, and were in accordance with National Institutes of Health Guide for the Care and Use of laboratory (permit No. 00117533).

MC-LR (purity >95%) standards purchased from Sigma Chemical (St. Louis, MO, U.S.A.) were dissolved in 0.9% saline solution at the desired concentrations. Treatment groups received intraperitoneal injections of either 5 or 20 μg MC-LR kg^{-1} body weight (bw) per day (low and high dose, respectively) for 4 weeks. The control group was treated with the same volume of 0.9% saline solution.

2.2. Physiological activity

Body weight, food consumption, water intake and the behaviors of each mouse were monitored for 4 weeks. At the end of experiment, mice were anesthetized with 50 mg kg^{-1} bw sodium pentobarbital. Blood was collected by cardiac puncture with a syringe containing EDTA for plasma biochemistry and thyroid hormone analysis. The brain and liver tissue of each mouse were also collected immediately, weighed, and transferred into 2-mL microcentrifuge tubes and stored in liquid nitrogen during necropsy. We then transferred the frozen tissue samples into a −80 °C freezer for the following analyses.

2.3. Blood biochemistry

Plasma thyroid-stimulating hormone (TSH), FT_3 and FT_4 were measured using mice ELISA kits purchased from Uscnlife (Wuhan, China). Total plasma triglyceride (TG), cholesterol (CHOL), high density lipoprotein cholesterol (HDL-c), and low density lipoprotein cholesterol (LDL-c) concentrations were measured by colorimetric methods using commercial kits (Nanjing Jian Cheng Bioengineering Co.). We measured glucose (GLU) levels in blood obtained from the tail vein using an Accu-check compact glucometer (Roche Diagnostic GmbH, Mannheim, Germany).

2.4. Glucose tolerance tests

To assess glucose tolerance and glucose clearance rate at the end of the experiment, mice were starved overnight for 12 h, and blood glucose was determined before and 30, 60, and 120 min following glucose (purity≥99.5%, Sigma) administration (2.0 g/kg bw glucose by intraperitoneal injections). Tests were performed in six mice.

2.5. Gene expression

Total RNA was extracted from the liver tissue using Trizol Reagent (Invitrogen) according to the manufacturer's instruction. Reverse transcription was conducted with 1 μg of total RNA from each sample, using an iScriptTM cDNA Synthesis Kit (BIORAD, Hercules, CA, USA). Quantitative real-time PCR reactions were performed with SYBR Green PCR kits (Toyobo, Tokyo, Japan) using an ABI 7900 system (Perkin Elmer Applied Biosystems, Foster City, CA, USA). Gene names, and forward and reverse primer sequences are listed in Table 1. Each sample was analyzed individually and processed in triplicate. The amount of target mRNA was normalized relative to that of *gapdh*. After verifying that the amplification efficiencies of the selected genes and *gapdh* were approximately equal, differences in expression levels were calculated using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001).

2.6. Western blot analysis

Brain tissues were lysed in 200 μL of RIPA buffer (50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 150 M NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM PMSF, 10 μg/mL aprotinin, and 10 μg/mL leupeptin).