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# Microcystin-LR altered mRNA and protein expression of endoplasmic reticulum stress signaling molecules related to hepatic lipid metabolism abnormalities in mice

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ARTICLE INFO

*Article history:*
Received 28 October 2014
Received in revised form
27 April 2015
Accepted 1 May 2015
Available online 11 May 2015

*Keywords:*
Microcystin-LR
Endoplasmic reticulum stress
Lipid metabolism abnormality
Liver
Toxic effect

ABSTRACT

To explore the effects of microcystin-LR (MC-LR), a hepatotoxin, on the incidence of liver lipid metabolism abnormality, and the potential molecular mechanisms of action, healthy male Balb/c mice were intraperitoneally injected with MC-LR at doses of 0, 5, 10, and 20 μg/kg/d for 14 days. Hepatic histopathology and serum lipid parameters of mice were determined, and the changes of mRNA and protein expression of endoplasmic reticulum (ER) stress signaling molecules related to the lipid metabolism abnormalities in the livers of mice were investigated by quantitative real-time polymerase chain reaction (qPCR) and Western blotting, respectively. The results indicated that 5–20 μg/kg/d MC-LR altered serum lipid parameters and caused hepatic steatosis. MC-LR treatment at 10 or 20 μg/kg/d changed mRNA and protein expression of ER stress signaling molecules, including upregulation of mRNA and protein expression of activating transcription factor 6 (ATF6), pancreatic ER eukaryotic translation initiation factor 2α (eIF-2α) kinase (PERK), and eIF-2α. MC-LR exposure at 10 or 20 μg/kg/d also altered mRNA and protein expression of downstream factors and genes of ER stress signaling pathways, including the downregulation of sterol regulatory element binding protein 1c (SREBP-1c) and fatty acid synthase (*FASn*), and upregulation of acetyl-coenzyme A carboxylase α (*ACACA*) and glycogen synthase kinase 3β (*Gsk-3β*). Our results reveal that ER stress plays a significant role in hepatic lipid metabolism abnormalities in mice exposed to MC-LR.



## 1. Introduction

Microcystins (MCs) are the most commonly found cyclic peptide hepatotoxins produced by several genera of freshwater cyanobacteria and they are extensively distributed in freshwater used as a source for drinking and recreational activities (Chorus et al., 2000). Currently, over 90 MC structural variants have been identified, among which MC-LR is one of the most toxic variants frequently reported to occur in lakes or

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http://dx.doi.org/10.1016/j.etap.2015.05.002
1382-6689/

reservoirs (Ufelmann et al., 2012). MC-LR is a very potent serine/threonine protein phosphatase 1 (PP1) and 2A (PP2A) inhibitor, and can covalently or non-covalently bind to the active site of the protein phosphatase and inhibit phosphatase activity (Craig et al., 1996). It has been reported that MC-LR can induce reactive oxygen species generation and cause oxidative stress in cell or animal models (Bouaicha and Maatouk, 2004; Ding et al., 2000). MC-LR can also cause many serious public health problems, including various liver diseases such as hepatitis, primary liver cancer, liver lipid peroxidation, and chronic liver injury (Dittmann and Wiegand, 2006; Levesque et al., 2014). Recently, He et al. (2012) have also indicated that MC-LR can affect hepatic lipid metabolism and induce hepatic lipid metabolism abnormalities, but the potential mechanism of action remains unclear.

The endoplasmic reticulum (ER) is a vital organelle that regulates fatty acid synthesis, cholesterol metabolism and protein folding, and plays an important role in producing lipids and secretory proteins (Babour et al., 2010). Various pathophysiological stimuli, such as hypoxia, glucose deprivation, inhibition of glycosylation, calcium depletion, viral infection, and perturbations in metabolic and energy balance, can cause an accumulation of unfolded and/or misfolded proteins in the ER, and can induce ER stress (Hotamisligil, 2010). During ER stress, the principal pathways of pancreatic ER eukaryotic translation initiation factor (eIF)-2a kinase (PERK), inositol-requiring enzyme (IRE)-1, and activating transcription factor 6 (ATF6) are activated, and each pathway culminates in transcriptional/post-transcriptional regulation of gene expression, which may contribute to homeostatic maintenance (Wek and Cavener, 2007). The activated pathways are also related to the occurrence of hepatic lipid metabolism abnormalities (Ozcan et al., 2004).

It has been demonstrated that the conditions generated under ER stress facilitate the activation of sterol regulatory element binding proteins (SREBPs) (Lee and Ye, 2004). The SREBP family of transcription factors is located in the ER and may include critical signaling molecules and mediators of the integration of lipid metabolism during the ER stress. The SREBP-1c isoform present in the majority of tissues, including liver tissue, is preferentially responsible for the regulation of target genes involved in fatty acid biosynthesis and cholesterol metabolism pathways (Gregor and Hotamisligil, 2007). The target genes include acetyl-coenzyme A carboxylase α (*ACACA*), fatty acid synthase (*FASn*), and glycogen synthase kinase 3β (*Gsk-3β*) (Colgan et al., 2011). Our previous study has shown that chronic MC-LR exposure can induce hepatocyte apoptosis via ER stress (Qin et al., 2010), and ER stress is also closely related to a lipid metabolism disorder (Rutkowski et al., 2008). However, little information is available about the effects of MC-LR on the occurrence of lipid metabolism abnormalities in mammals via ER stress.

This study aimed to investigate the effects of MC-LR on hepatic lipid metabolism of mice, the roles of ER stress signaling molecules in the hepatic lipid metabolism disturbance, and the underlying toxicological mechanisms related to ER stress. Therefore, in the present study, hepatic histopathology and serum metabolic parameters of mice were determined after the mice were exposed to MC-LR by intraperitoneal injection for 14 days. Quantitative real-time polymerase chain reaction (qPCR) and Western blotting were used to investigate the effects of MC-LR on mRNA and protein expressions of ER stress signaling molecules and on downstream factors and genes of ER stress signaling pathways related to lipid metabolism in the livers of mice.

## 2. Materials and methods

### 2.1. Chemicals

MC-LR (purity ≥95%, as assessed by HPLC) purchased from Alexis Biochemicals (Lausen, Switzerland) was dissolved in the minimal amount of dimethyl sulfoxide (DMSO) (Amresco, Solon, OH, USA) (0.1%), and then diluted to the required concentration using sterile saline. Final concentration of DMSO in the MC-LR solution was less than 0.01%. Unless otherwise mentioned, all other chemicals were obtained from Sigma–Aldrich Inc. (St. Louis, MO, USA).

### 2.2. Animals

All animal experiments were performed according to the guidelines of the Animal Care and Use Committee of Nanjing University and in accordance with the protocols approved by the Care and Use of Laboratory Animals (National Research Council, Helsinki, Finland). Healthy male Balb/c mice (6 weeks old and 18–22 g weight) used in this study were obtained from the Chinese Academy of Medical Sciences (Beijing, China), and housed on wood shavings in plastic cages with five mice per cage. Mice were acclimatized to laboratory conditions for one week prior to exposure experiments, and maintained in temperature (21 ± 0.3 °C) and humidity (60 ± 5%) controlled rooms with standard lighting conditions (12-h light:12-h dark cycle) during experiments.

### 2.3. Mice exposed to MC-LR

A total of 40 mice were divided into four groups (10 mice in each group) and were exposed to different doses of MC-LR (0, 5, 10, and 20 μg/kg/d). In the treatment groups, Balb/c mice were injected intraperitoneally with 200 μL of the different doses of MC-LR daily for 14 days. In the control group (0 μg/kg/d MC-LR), the mice were exposed to 200 μL of 0.9% sterile saline solution by intraperitoneal injection daily for 14 days.

### 2.4. Body and liver weights of mice

The initial and final body weights were recorded on the first day and the 14th day, respectively. All the mice were sacrificed on the 15th day. The liver was eviscerated, washed in normal saline, blotted, weighed (absolute weight), immediately frozen in liquid nitrogen, and then stored at −80 °C until further analysis. The relative liver weight was calculated as a ratio of liver to body weight of each mouse.