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MC-LR induces dysregulation of iron homeostasis by inhibiting hepcidin expression: A preliminary study

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HIGHLIGHTS

- MC-LR induced the accumulation of iron in C57BL/6 mice with the occurrence of anemia that similar to thalassemia.
- MC-LR reduced the expression of hepcidin that is the central hormone in regulating iron homeostasis in the body.
- The downregulation of hepcidin induced by MC-LR may be related to hypoxia, IL-6-STAT3, and BMP-SMAD signaling pathway and HIFs play important roles.

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ABSTRACT

The liver is an important iron storage site and a primary MC-LR target. C57BL/6 and *Hfe*$^{-/-}$ mice were used to investigate effects and mechanisms of MC-LR on systematic iron homeostasis. Body weight, tissue iron content, hematological and serological indexes, and histopathological were evaluated. Ultrastructure and iron metabolism-related genes and proteins were analyzed. MC-LR induced dose-dependent increases in red blood cells, hemoglobin, and hematocrit. In contrast MC-LR-induced dose-dependent decreases in mean corpuscular volume, hemoglobin, and hemoglobin concentration were observed both C57BL/6 and *Hfe*$^{-/-}$ mice. In both mouse species, serological indexes increased. Aggravated liver and spleen iron were observed in C57BL/6 mice, consistent with Perls' Prussian blue staining. However, an opposite trend was observed in *Hfe*$^{-/-}$ mice. C57BL/6 mice had lower *Hamp1* (*Hepcidn*), *Bmp6*, *Il-6*, and *Tmprss6*. Significant increased *Hjv*, *Hif-1α* and *Hif-2α* were observed in both C57BL/6 and *Hfe*$^{-/-}$ mice. MC-LR-induced pathological lesions were dose-dependent increase in C57BL/6 mice. More severe pathological injuries in MC-LR groups (25 μg/kg) were observed in *Hfe*$^{-/-}$ mice than in C57BL/6 mice. In *Hfe*$^{-/-}$ mice, upon exposure to 25 μg/kg MC-LR, mitochondrial membranes were damaged and mitochondrial counts increased with significant swelling. These results indicated that MC-LR can induce the accumulation of iron in C57BL/6 mice with the occurrence of anemia, similar to thalassemia. Moreover, dysregulation of iron homeostasis may be due to MC-LR-induced *Hamp1* downregulation, possibly mediated by hypoxia or the IL6–STAT3 and BMP–SMAD signaling pathways.

1. Introduction

Microcystins (MCs), a cyclic heptapeptide produced by cyanobacteria in eutrophic waters, are potent hepatotoxins, with the general structure of cyclo (-D-Ala1-L-X2-D-erythro-β-methylAsp3-L-Z4-Adda5-D-Glu6-N-methyldehydeo-Ala7) (Svircev et al., 2010; Chen et al., 2015). More than 100 MC structural variants have been identified so far (Chen et al., 2015, 2018), of which microcystin-leucine arginine (MC-LR) is the most widespread and virulent type. MC-LR poses a threat to humans in drinking water, contaminated aquatic products and edible crops irrigated by MC-LR-contaminated water (Lee et al., 2017; Hu et al., 2018). MC-LR can result in hepatotoxicity, cardiotoxicity, nephrotoxicity,

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neurotoxicity, and spleen toxicity (Milutinovic et al., 2006; Svircev et al., 2010; Piyathilaka et al., 2015; Zhao et al., 2015; Lone et al., 2016) and has attracted worldwide attention for its toxicological consequences. Since the 90's, the World Health Organization has established a hygienic standard of 1.0 μg/L of MC-LR levels in drinking water (WHO, 1998).

The liver is the primary target organ of MC-LR via the specific transporters OATP1B1 and OATP1B3 (Feurstein et al., 2010; Steiner et al., 2016; Teneva et al., 2016). Further, the activity of protein phosphatase 2A (PP2A) and protein phosphatase 1 (PP1) are inhibited in the liver after exposure to MC-LR, resulting in excessive protein phosphorylation, microfilament decomposition, cell rupture, and hemorrhage (Svircev et al., 2010; Ma et al., 2018a, b). MC-LR induces hepatic oxidative damage, lipid peroxidation, cytoskeletal disruption, and even hepatocyte apoptosis (Sedan et al., 2015; Zhou et al., 2015; Zhang et al., 2016; Martins et al., 2017; Ma et al., 2018a; b). Epidemiological survey results showed that high MC-LR-exposed children had significantly higher levels aspartate aminotransferase (AST) and alkaline phosphatase (ALP), indicators of liver damage, compared to children with low MC-LR-exposure or unexposed children (Li et al., 2011). Exposure to chronic low-dose MC-LR can increase the risk of liver cancer (Zegura et al., 2003).

Animal experiments have shown that MC-LR is a liver tumor promoter (Nishiwaki-Matsushima et al., 1992) consistent with human epidemiological surveys (Svircev et al., 2009). The mechanisms of MC-LR-induced hepatotoxicity have not been fully elucidated but it is known that iron overload can result in liver damage (Bao et al., 2016). Hepatocytes, a main storage site for trace iron elements, are the most predominant cell types in the liver. Liver injury and iron homeostasis are closely related.

MC-LR can also induce lesions in the heart by enlarging cardiomyocytes, destroying cell cross-striations, lower myofibril volume fraction, myocardial fibrosis, and mononuclear infiltration in the interstitial tissue (Milutinovic et al., 2006). The blood circulated by the heart supplies nutrients to the whole body and iron is an essential microelement of hemoglobin; consequently, MC-LR-induced lesions in the heart may induce hypoxia as hemoglobin delivers oxygen from the lungs throughout the body resulting in systemic toxicological effects.

Studies in the human embryonic kidney cell line (HEK-293) and the human kidney adenocarcinoma cell line (ACHN) demonstrate that 24 h exposure to pure MC-LR (1.0–200 μM) results in significant cell viability decreases and upregulation of apoptosis-related genes, including Bax, Survivin, and p53, and proteins, including caspase 3 and caspase 9 (Piyathilaka et al., 2015). These findings indicate that MC-LR has the potential to induce kidney damage. MC-LR-induced liver and kidney damage may lead to iron-eliminating disorders, resulting in the accumulation of iron in the body.

MC-LR can activate neutrophils and macrophages and induces splenomegaly, leading to the production of reactive oxygen species (ROS) that damage and injure the spleen and alter immune correlating factors, such as NO, chemokines, IL-1β, and TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Lone et al., 2016). Iron recycling plays an important part in maintaining systematic iron homeostasis. Approximately 0.5–2.0 mg of iron is derived from food and absorbed by the small intestine. However, most of the iron in the body is derived from recycled heme iron after phagocytosis of senescent erythrocytes by macrophages. In macrophages, hemoglobin iron is released by heme-oxidase (HO-1), and Fe^{2+} is transported to the blood by ferroportin-1 (FPN1) and ceruloplasmin to complete the recycle process (Poss and Tonegawa, 1997; Donovan et al., 2000; Delaby et al., 2005). MC-LR-induced activation of macrophages affects the immune system and may disturb systematic iron homeostasis.

MC-LR also changes the cerebrum ultrastructure, leading to distention of the endoplasmic reticulum and swelling of the mitochondria (Zhao et al., 2015). Moreover, MC-LR can cause the dysfunction of astrocytic homeostatic capabilities and induce cytoskeletal disruption (Zhao et al., 2015). Central nervous system cells, particularly neurons, are metabolically exuberant cells that require high iron levels. Systematic iron level plays an important role in maintaining neurocyte function. Studying the effect of MC-LR on systematic iron may establish a foundation for protecting neurological diseases.

Iron, an essential microelement, with the capacity to accept and donate electrons in some fundamental biochemical reactions, such as oxygen transportation, redox reaction, and cellular respiration, is an indispensable component of cytochromes, hemoglobin, myoglobin, and multiple enzymes. Therefore, iron homeostasis plays an important role in metabolic balance (Feng et al., 2011). Blockage of the routes of systematic iron circulation may be lethal. Hepcidin (HAMP1), a 25 amino acid peptide hormone produced by hepatocytes, is the key regulator of iron metabolism via binding to the cellular iron exporter FPN, the only known mammalian cellular iron exporter, triggers its internalization and degradation in lysosomes, which consequently suppresses iron uptake from diet and iron release from macrophages after phagocytosis of senescent erythrocytes (Gnana-Prakasam et al., 2014; Ravasi et al., 2014; Alkhateeb et al., 2015). Thus, *Hamp1* expression can significantly affect the extent of iron load in the main iron storage organs, such as liver, and can determine circulating iron levels in the body.

Hamp1 is a key regulator of iron metabolism. It was decreased in the C57BL/6 mice after exposure to MC-LR. $Hfe^{-/-}$ mice (129/SvEvTac background) are iron storage disease hereditary hemochromatosis (HH) mice, whose pathophysiology is characterized by decreased production and activity of *Hamp1* (Miranda et al., 2003). Therefore, $Hfe^{-/-}$ mice were used to observe whether *Hamp1* were further decreased and confirm whether the dysfunction of iron homeostasis is disturbed by the decrease of *Hamp1* induced by MC-LR. At the same time, the expression of relevant factors and proteins in regulation of hamp1 in $Hfe^{-/-}$ mice were detected. Iron accumulation can cause damage to various tissues. C57BL/6 mice exhibit a state of iron accumulation after exposed to MC-LR. Another characteristic of $Hfe^{-/-}$ mice is progressive iron accumulation in various tissues, particularly in the liver (Miranda et al., 2003). It was speculated that $Hfe^{-/-}$ mice showed more serious damage to liver compared that to C57BL/6 mice in same dosage of MC-LR. Therefore, $Hfe^{-/-}$ mice were used to observe whether the liver damage caused by iron accumulation was aggravated after exposure to MC-LR. $Hfe^{-/-}$ mice are often used for studying iron metabolism-related diseases (Miranda et al., 2003) and mechanisms in regulation of iron homeostasis (Wang et al., 2017). This study is the first to use $Hfe^{-/-}$ mice for MC-LR research.

MC-LR can induce injures in multiple organs, such as the liver, heart, spleen, and kidney. These organs play a significant role in maintaining iron metabolism. MC-LR-induced tissue damage may disturb systematic iron homeostasis. The goal of this study was to explore the mechanisms of MC-LR-induced imbalance in iron homeostasis, to provide potential preventive and therapeutic targets for iron homeostasis-related diseases, and to enrich the biological function of microcystin and metabolic theory of microelement.

2. Materials and methods

2.1. Reagents

MC-LR, purity >96%, was purchased from Beijing Express Technology Co. (Beijing, China). 1 μg/kg MC-LR powder was