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Review

Mechanisms of microcystin-LR-induced cytoskeletal disruption in animal cells

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ABSTRACT

Microcystin-LR (MC-LR), a potent hepatotoxin produced by certain bloom-forming cyanobacteria, covalently binds to serine/threonine protein phosphatases and acts as an efficient inhibitor of this group of enzymes. MC-LR induces oxidative stress and the unfolded protein response in multiple cell types, leading to apoptosis through the mitochondrial and endoplasmic reticulum pathways. Histologic lesions of acute MC-LR toxicosis exhibit membrane blebbing, cell rounding and dissociation, indicating that this toxin may exert hepatotoxic effects by causing cytoskeletal disruption. Both *in vivo* and *in vitro* studies have revealed that exposure of human, mouse, or rat hepatocytes to MC-LR induces the rearrangement or collapse of the three components of the cytoskeleton. In addition, multiple cytoskeletal and cytoskeleton-associated proteins have been found to be affected by MC-LR. This review summarizes the increasing information in the literature pertaining to the molecular mechanisms of MC-LR-induced cytoskeletal disruption and may increase our understanding of its toxicity.

1. Introduction

Cyanobacteria are photoautotrophic prokaryotic organisms that are commonly found in freshwater, in marine systems, and in symbiosis with plants as well. They mainly proliferate in lakes and rivers where eutrophication has resulted from increases in human population and the consequent intensification of agricultural and industrial activities along with deficient water management (de Figueiredo et al., 2004). Specific environmental conditions, such as higher temperature and pH levels, low turbulence, and high nutrient inputs (especially phosphorus and nitrogen), enhance the growth of cyanobacteria in various types of freshwater, leading to the formation of surface blooms (Campos and Vasconcelos, 2010; de Figueiredo et al., 2004). Cyanobacterial growth is a natural phenomenon, but the development of cyanobacterial blooms has become more frequent and severe in the past decades. One particularity of cyanobacteria is the production of secondary metabolites that are toxic to many organisms, including humans (Corbel et al., 2014; de Figueiredo et al., 2004; Dittmann et al., 2013; El Khalloufi et al., 2013; Mulvenna et al., 2012). Cyanobacterial blooms produce excessive toxins that can accumulate in aquatic wildlife and be transferred to higher trophic levels, leading to an increased risk of human poisoning (Dietrich et al., 2008; Paerl, 2014).

Cyanotoxins, which are produced by cyanobacteria, are very diverse in their chemical structures and toxicities (Ferrao-Filho Ada and Kozlowsky-Suzuki, 2011; Merel et al., 2013; Zanchett and Oliveira-Filho, 2013) and can be classified as hepatotoxins (microcystins and nodularins), neurotoxins (anatoxin-a, anatoxin-a(S), homoanatoxin-a, and saxitoxins) and dermatotoxins (aplysiatoxins, lipopolysaccharides, and lyngbyatoxin-a), according to their toxic effects on animals (de Figueiredo et al., 2004). Microcystins (MCs) comprise a family of hepatotoxins named after the first organism identified as producing them, *Microcystis aeruginosa*. It was later found that they can also be produced by other genera, namely *Anabaena*, *Aphanizomenon*, *Nostoc*, *Oscillatoria* and *Planktothrix* (Dawson, 1998; van Apeldoorn et al., 2007).

MCs are monocyclic heptapeptides, with the general structure cyclo-(D-Ala^1-X^2-DMeAsp^3-Z^4-Adda^5-D-Glu^6-Mdha^7). The structure consists of a D-alanine at position 1, two variable L-amino acids at positions 2 (X) and 4 (Z), and 3 unusual amino acids: β-linked D-*erythro*-β-methylaspartic acid (DMeAsp) at position 3; (2*S*,3*S*, 8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) at position 5; γ-linked D-glutamic acid at position

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6, and *N*-methyl dehydroalanine (Mdha) at position 7 (Dawson, 1998; McElhiney and Lawton, 2005; Sivonen et al., 1992; van Apeldoorn et al., 2007) (Fig. 1). More than 80 variants of MCs have been identified in freshwater systems, which differ primarily in the two L-amino acids at positions 2 and 4 (Hoeger et al., 2007). Other structural variations have been attributed to the methylation/demethylation of DMeAsp and Mdha at positions 3 and 7 (Maizels and Budde, 2004). The unusual amino acid Adda is highly conserved in MCs and is stable against physiological replacement with other amino acids. Due to this stability, the Adda group is typically utilized in various methods for the identification of microcystins (Msagati et al., 2006). Previous study has also shown that the Adda region is crucial for interactions with protein phosphatases; thus, it is important for the toxicity of MCs (Barford and Keller, 1994). The most common MCs are MC-LR, MC-RR and MC-YR, which contain different combinations of leucine (L), arginine (R) or tyrosine (Y) at positions 2X and 4Z. Among 80 + variants, MC-LR is the most studied owing to its ubiquity, abundance and toxicity (Hoeger et al., 2005; Zurawell et al., 2005).

2. The toxicity of MC-LR

MC-LR has drawn special attention due not only to its ability to cause acute poisoning but also to its cancer-promoting potential in humans following chronic exposure to low concentrations in drinking water. Therefore, the World Health Organization has established that the guideline value of MC-LR in drinking water is 1 μg/L (Chorus and Bartram, 1999). Although the liver is the primary target organ of MC-LR as the result of a hepatocyte-specific organic anion transporting polypeptide (OATP) membrane transport system that carries it into hepatocytes (Clark et al., 2007; Fischer et al., 2005), it can also affect some other organs, such as the brain (Kist et al., 2012), heart (Milutinovic et al., 2006), intestine (Zegura et al., 2008a), kidney (Qin et al., 2010), and reproductive organs (Wu et al., 2014).

2.1. MC-LR inhibits protein phosphatases (PPs)

The most studied toxic mechanism of MC-LR is the inhibition of serine/threonine protein phosphatases by interactions with the catalytic subunits of PPs (Maynes et al., 2006). Protein phosphorylation/dephosphorylation, catalyzed by phosphatases and kinases, are dynamic processes and important manners of regulating the protein activity in cells. Therefore, the inhibition of these enzymes may have significant impacts on cellular homeostasis.

MC-LR has strong affinities for PP1 and PP2A but has little effect on PP2B (Honkanen et al., 1990). PP2A is a major protein phosphatase in cells that is involved in diverse processes, including cell proliferation and death, cell mobility, cytoskeleton dynamics, the control of the cell cycle, and the regulation of numerous signaling pathways (Janssens and Goris, 2001; Lechward et al., 2001; Shi, 2009; Sontag, 2001). Cellular PP2A exists in two general forms, a heterodimeric core enzyme and a heterotrimeric holoenzyme. The PP2A core enzyme consists of a 65-kDa scaffold subunit (also known as the A or PR65 subunit) and a 36-kDa catalytic subunit (C subunit). The scaffold and catalytic subunits each have two isoforms, α and β, and the α isoform is approximately 10-fold more abundant than the β isoform. The PP2A core enzyme interacts with a variable regulatory subunit (B subunit) to assemble into a holoenzyme and thus determines the specific functions of PP2A (Cho and Xu, 2007; Li et al., 2011b) (Fig. 2). PP2A/C is a major target of numerous toxins, possibly because it is one of the most evolutionarily conserved enzymes and is a major cellular serine/threonine protein phosphatase in eukaryotic cells (MacKintosh et al., 1990). In a human normal liver cell line (HL7702), PP2A activity has been shown to decrease in a concentration-dependent manner after MC-LR treatment. In addition, exposure to this toxin causes the phosphorylated PP2A/C to significantly increase and that of methylated PP2A/C to slightly decrease. Following treatment with a high concentration of MC-LR (10 μM), the protein levels of PP2A/A and B55α have been shown to increase slightly (Sun et al., 2014).

As the most potent PP2A inhibitor, MC-LR covalently binds to PP2A/C at Cys269 269 and directly and/or potently inhibits its catalytic activity, affecting numerous signaling pathways (Perrotti and Neviani, 2013). Therefore, its toxicity can be assessed by determining the functions of PP1 and PP2A and their protein substrates (Campos and Vasconcelos, 2010). Moreover, the well-known effects of MC-LR on DNA damage, cytoskeletal disruption, cell cycle arrest, and cell death have been related to PP1/PP2A activity and the increased phosphorylation of certain proteins (Chen et al., 2012; Meng et al., 2011; Sun et al., 2011b; Zeng et al., 2014), indicating the importance of PPs in MC-LR-mediated acute/chronic toxicity.

2.2. MC-LR induces oxidative stress

A biochemical feature of MC-LR toxicity is the induction of oxidative stress through an increase in the formation of reactive oxygen species (ROS) and/or the depletion of glutathione (GSH), leading to cellular dysfunction and apoptosis. ROS-mediated MC-LR toxicity has been detected using *in vitro* systems, such as a human hepatoma cell line (HepG2) (Shi, 2009), neuroendocrine cell line (PC12) (Meng et al., 2015), human colon carcinoma cell line (Caco-2) (Puerto et al., 2010), primary cultured rat hepatocytes (Ding et al., 2000b), and human erythrocytes (Sicinska et al., 2006), and it has also been reported by many *in vivo* studies of the liver, heart and reproductive system (Chen et al., 2014; Li et al., 2008; Qiu et al., 2009; Sun et al., 2011a; Weng et al., 2007).

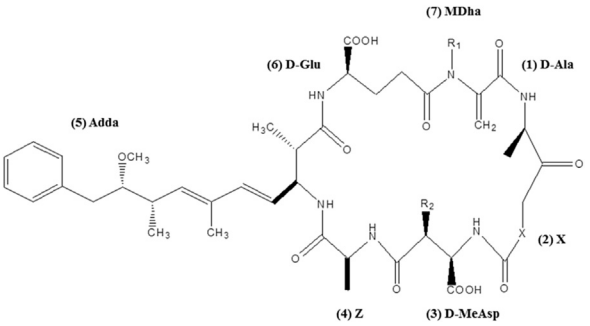

Fig. 1. General structure of MCs. For MC-LR, X represents L-Leucine, and Z represents L-Arginine.

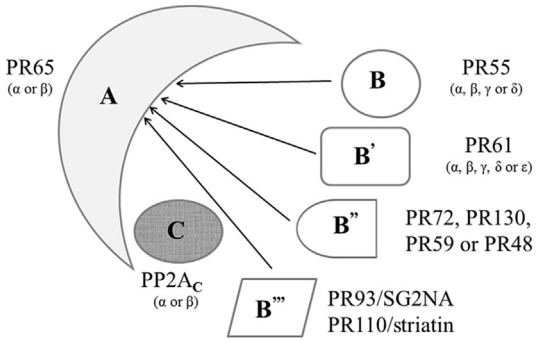

Fig. 2. The molecular structure of PP2A.