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Mechanisms of Microcystin-induced Cytotoxicity and Apoptosis

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Abstract: In recent years, cyanobacterial blooms have dramatically increased and become an ecological disaster worldwide. Cyanobacteria are also known to produce a wide variety of toxic secondary metabolites, *i.e.* cyanotoxins. Microcystins (MCs), a group of cyclic heptapeptides, are considered to be one of the most common and dangerous cyanobacterial toxins. MCs can be incorporated into the cells via organic anion transporting polypeptides (Oatps). It's widely accepted that inhibition of protein phosphatases (PPs) and induction of oxidative stress are the main toxic mechanisms of MCs. MCs are able to induce a variety of toxic cellular effects, including DNA damage, cytoskeleton disruption, mitochondria dysfunction, endoplasmic reticulum (ER) disturbance and cell cycle deregulation, all of which can contribute to apoptosis/programmed cell death. This review aimed to summarize the increasing data regarding the intracellular biochemical and molecular mechanisms of MC-induced toxicity and cell death.

Keywords: Apoptosis, cell death, cytoskeleton, genotoxicity, microcystin, mitochondria, oxidative stress, protein phosphatase.

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1. INTRODUCTION

Cyanobacteria, commonly called blue-green algae, are evolutionarily ancient prokaryotic, Gram-negative, and oxygenic photosynthetic microorganisms found ubiquitously in nature. Cyanobacteria are known as one of the primitive oxygen-producing organisms on Earth with the fossil records of about 3.5 billion years [1]. As a result of their very long evolutionary history, the cyanobacteria have gained a lot of special abilities, including fixing atmospheric nitrogen, solubilizing phosphorous and sequestering iron. These make them successfully adapted to almost all the terrestrial and aquatic environments on which life can exist [2]. However, cyanobacterial blooms, characterized by excessive proliferation of cyanobacterial cells, have dramatically increased due to water eutrophication and global warming and become an ecological disaster worldwide in recent years (Fig. **1**). Cyanobacteria are notorious for their production of unpleasant tastes and odors and toxic cyanotoxins. Cyanotoxins are a diverse group of secondary metabolites produced by various genera of cyanobacteria and are toxic to eukaryotic organisms including algae, plants, animals and even humans.

According to their chemical composition, cyanotoxins are divided into three groups, *i.e.*, cyclic peptides (microcystins and nodularins), alkaloids (anatoxin-a, anatoxina (s), saxitoxins, cylindrospermopsin, aplysiatoxin, lyngbiatoxin-a) and lipopolysaccharides (LPSs) [3]. Among all the cyanobacterial toxins, microcystins (MCs) are considered to be one of the most common and dangerous groups. The occurrence of MCs have been reported in several cyanobacterial genera such as *Anabaena*, *Anabaenopsis*, *Aphanocapsa*, *Aphanizomenon*, *Cylindrospermopsis*, *Fischerella*, *Hapalosiphon*, *Lyngbya*, *Microcystis*, *Nostoc*, *Oscillatoria* (*Planktothrix*), *Phormidum*, *Rivularia* and *Synechococcus* but most frequently in the species of *Anabaena* as well as *Microcystis* (reviewed in [3]). MCs are small molecular weight compounds of approximately 1000 Da with over 100 structural variants having been identified to date, and MC-LR, MC-RR, and MC-YR, with different combinations of leucine (L), arginine (R) or tyrosine (Y), are the most widely studied due to their ubiquity, abundance and toxicity (Fig. **2**) [4]. They contain seven peptide-linked amino acids, with the two terminal amino acids of the linear peptide being condensed (joined) to form a cyclic compound, *i.e.* monocyclic heptapeptides. MCs share a general structure, cyclo-(-D-Ala^1-L-X^2-D-isoMeAsp^3-L-Z^4-Adda^5-D-isoGlu^6-Mdha^7), where D-isoMeAsp is D-erythro-β-methyl-aspartic acid, Mdha is N-methyl-dehydro-alanine, and X and Z in positions two and four are highly variable L-amino acids that determine the suffix in the nomenclature of MCs [3]. For example, microcystin-LR (MC-LR) contains leucine (L) and arginine (R), whereas microcystin-YA (MC-YA) contains tyrosine (Y) and alanine (A). Adda is an unusual C20 amino acid (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4E,6E-dienoic acid, which is found only in cyanobacterial peptides (MCs and nodularins, NODs) and is crucial for the interactions with protein phosphatases (PPs), making this region important for the toxicity of these cyanotoxins. The most severe human toxicity event happened

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Fig. (1). Microcystis aeruginosa bloom in Lake Chaohu, China. (Photographed by Mr. Wulai Xia).

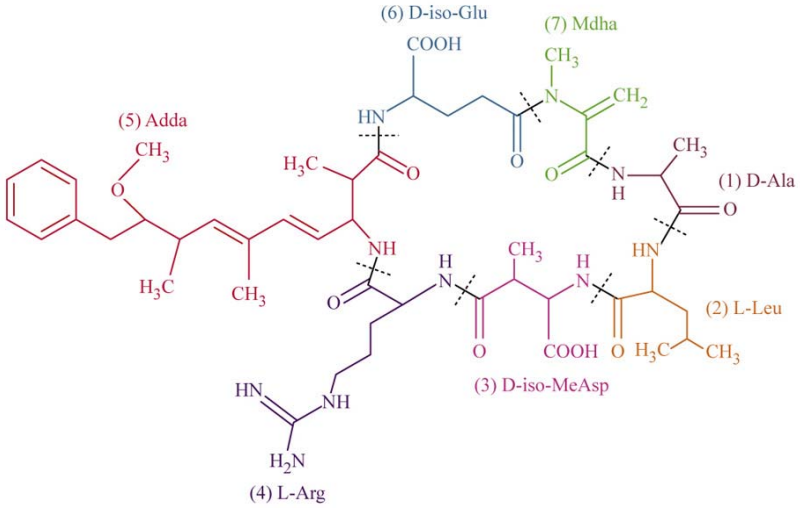

Fig. (2). Chemical structure of microcystin-LR (MC-LR).

in February 1996 in Brazil, where 100 of 131 patients developed acute liver failure due to MCs contamination of the water used for hemodialysis and 52 patients died [5].

The physico-chemical properties and stability of toxins may be critical for their ultimate toxicity. MCs are extremely stable and resist to common chemical breakdown such as hydrolysis or oxidation under conditions found in most natural water bodies, possibly because of their cyclic structure and novel amino acids [6]. At 40 °C, the half-life of MC-LR is about 3 and 10 weeks at pH 1 and 9, respectively [7]. MCs are also stable under irradiation by sunlight, although sunlight irradiation with pigments contained in cyanobacteria significantly decomposed the toxins by isomerization of a double bond in the Adda side chain; the half-life of photolysis was observed to be about 10 days [8, 9]. Rapid chemical hydrolysis and photolysis occur only under laboratory conditions that are unlikely to be attained outside the laboratory, *e.g.* 147 μW/cm^2 ultraviolet (UV) irradiation, 6 mol/L HCl at high temperature [6, 9]. In laboratory experiments using raw reservoir water containing low levels of MC-LR (10 μg/L), primary degradation of the toxin was shown to occur in less than 1 week. Further studies showed that MC-LR was stable over 27 days in deionised water, and over 12 days in sterilised reservoir water, indicating that the instability in normal reservoir water is due to biodegradation [10]. Although MCs can be broken down by some bacterial proteases, in many circumstances these bacteria are not always present in sufficient cell density or need to adapt so the toxin persists for months or even years once released into cooler, dark, natural water bodies. It has been reported that MCs can sometimes persist for relatively long time in natural environment, ranging from 1 to 3 months, to up to 6 months in dry scums [11]. Moreover, MCs can withstand several hours in boiling water and it has been suggested that cooking cannot completely remove MCs accumulated in aquatic animals [12-14]. The toxins are also resistant to enzymatic hydrolysis by some common digestive enzymes, such as pepsin, trypsin, and chymotrypsin. Thus, chemically stable MCs in natural water and aquatic products may cause severe health risks to animals and human beings.

2. CELLULAR UPTAKE AND SUB-CELLULAR DISTRIBUTION OF MCs

As a result of their structure and amino acid composition, MCs are rather hydrophilic and spatially large molecules, hindering their free/passive diffusion or penetration across the cell membrane [15]. Therefore, an active transport mechanism via specific transporters must be present in the cells through which MCs can be absorbed. The uptake of tritiated dihydro-microcystin-LR ([^{3}H]-2H-MC-LR) was shown to be specific for freshly isolated rat hepatocytes whereas the uptake in the human hepatocarcinoma HepG2 and neuroblastoma SH-SY5Y cell lines as well as the mouse fibroblast NIH-3T3 cell line, was negligible [16]. Moreover, it was shown that the membrane penetrating capacity (surface activity) of MC-LR was low (by a surface barostat technique), supporting that the toxin requires an active uptake mechanism. Furthermore, the hepatocellular uptake of MC-LR could be inhibited by bile acid transport inhibitors such as antamanide, bromosulfophthalein (BSP) and rifampicin (RFP), and by bile salts cholate and taurocholate (TC), indicating that the uptake of MC-LR occurs through the multispecific transport system for bile acids [16, 17]. This mechanism of cell entry would explain the organotropism and cell type specificity of MC-LR. The cyclosporin A (CsA), rifamycin, trypan blue, trypan red, cholate, TC and BSP also decreased the accumulation of ^{125}I labeling of MC-YM and the inhibition of protein phosphatases (PPs) in rat hepatocytes [18]. Uptake of [^{3}H]-2H-MC-LR was rapid in hepatocytes suspension and perfused livers at 37°C; however, uptake in cell suspensions was reduced by incubation of hepatocytes at 0 °C, suggesting that the uptake of MC-LR occurs primarily by an energy-dependent transport process [17]. In the hepatocyte suspensions, 65% to 77% of the radiolabel was in the cytosolic fraction, and 13% to 18% of the radiolabel was present in the plasma membrane/nuclear fraction, with lesser amounts in the other fractions. Moreover, trichloroacetic acid treatment of cytosolic fractions indicated that 50-60% of the radiolabel was bound to cytosolic protein [17]. Similar results were also observed in perfused rat liver *in vitro* [17] and in mouse liver *in vivo* [19].

Most uptake transporters which are responsible for the uptake of endogenous and exogenous chemicals into cells belong to the superfamily of solute carriers (SLCs), including the organic anion transporting polypeptides (Oatps), organic anion transporters (Oats), organic cation transporters (Octs), and Na-taurocholate cotransporting polypeptide (Ntcp) [20]. In fact, cholate and taurocholate, which were shown to inhibit the uptake of MCs by hepatocytes, are substrates of Oatps [21]. Using the *Xenopus laevis* oocyte expression system, Fischer *et al*. [21] showed that rat Oatp1b2, human OATP1B1 and human OATP1B3, which are expressed in rat/human hepatocytes, transported MC-LR 2- to 4-fold above water-injected control oocytes (Fig. **3**). Human brain OATP1A2-expressing oocytes were also able to accumulate more MC-LR compared with controls. MC-LR transport was inhibited by the known Oatp/OATP substrates taurocholate (TC) and bromosulfophthalein (BSP). However, no MC-LR transport was observed in oocytes expressing Oatp1a1, Oatp1a4 or OATP2B1. These results may explain some of the observed organ-specific toxicity of MC-LR [21]. Evidence that Oatp1b2-null mice displayed markedly decreased hepatic uptake and toxicity of MC-LR, demonstrated the essential role of Oatps/OATPs in MCs uptake and toxicity [22]. The binding of MC-LR to PP1/2A was attenuated, but not abolished, in livers of Oatp1b2-null mice, indicating that in addition to Oatp1b2-mediated transport, MC-LR can enter cells by passive diffusion or other mechanism(s) at a lower rate.

Beyond OATP1A2, other OATPs, such as Oatp1a5 and Oatp1b2 may be also responsible for the active transmembrane transport of MCs into the brain cells [23, 24]. Immunofluorescent analyses using confocal microscopy demonstrated the presence of MC-LR in the cytosol and the nuclei of brain cells. Co-incubation of TC and BSP with MCs (-LF, -LW and -LR) generally resulted in a reduction of MCs uptake and cytotoxicity [23]. MC variants transport by Oatps was also suggested in the mouse neuron uptake inhibition experiments, which showed that TC and estrone sulfate ammonium salt (ES) uptake in neurons was inhibited by MC-LR, MC-LW and MC-LF [24]. Because all of the Oatps known to transport MCs are also established transporters of TC and ES, it is not surprising that co-incubations of TC or ES with equimolar concentrations of MC-LR, MC-LW, and MC-LF reduced the uptake of TC and ES. MC-LR, MC-LW, and MC-LF have been reported to have comparable toxicodynamic properties (PPs inhibition), but MC-LF induced the greatest reduction in PPs activity after exposure of neuronal cells to equimolar concentrations of the three MC congeners, which suggests that MC-LF was transported more efficiently into the cell (toxicokinetics) and thus reached PP-inhibitive intracellular concentrations more quickly than did MC-LR and MC-LW [24]. However, only specific expression of individual Oatp and MC-transport analyses will allow determination of the contribution of each individual Oatp expressed in brain cells in the observed MC-congener specific transport [23].

Zeller *et al*. [25] reported a fast uptake of MC-LR and MC-RR in a human intestinal cell culture model (Caco-2 cells), with a similar profile for both variants. Indeed, after only 30 min of treatment, staining of MCs at the cell membrane could be clearly observed. After 2 h the toxins progressed inside the cell reaching the cytoplasm and the nucleus after 6 h, although the nuclear localization of MCs was weak. Caco-2 cells incubated with MC-LR or MC-RR at 4 °C showed a consistent staining similar for both toxin variants, suggesting that cellular uptake occurs through a facilitated diffusion rather than an ATP-dependent transfer. The localization of OATP3A1 and OATP4A1 at the cell membrane of Caco-2 cells may be involved in the uptake of MCs [25].

3. MC-INDUCED TOXICITY AND CELL DEATH

The effects of MCs on cellular metabolism and physiology vary according to species/genetic backgrounds, cell type, MC congeners, levels and duration of exposure. For example, the sensitivity of primary mouse hepatocytes toward MC-LR was about 25-fold greater than that of rainbow trout hepatocytes [26]. Ikehara *et al*. [27] reported

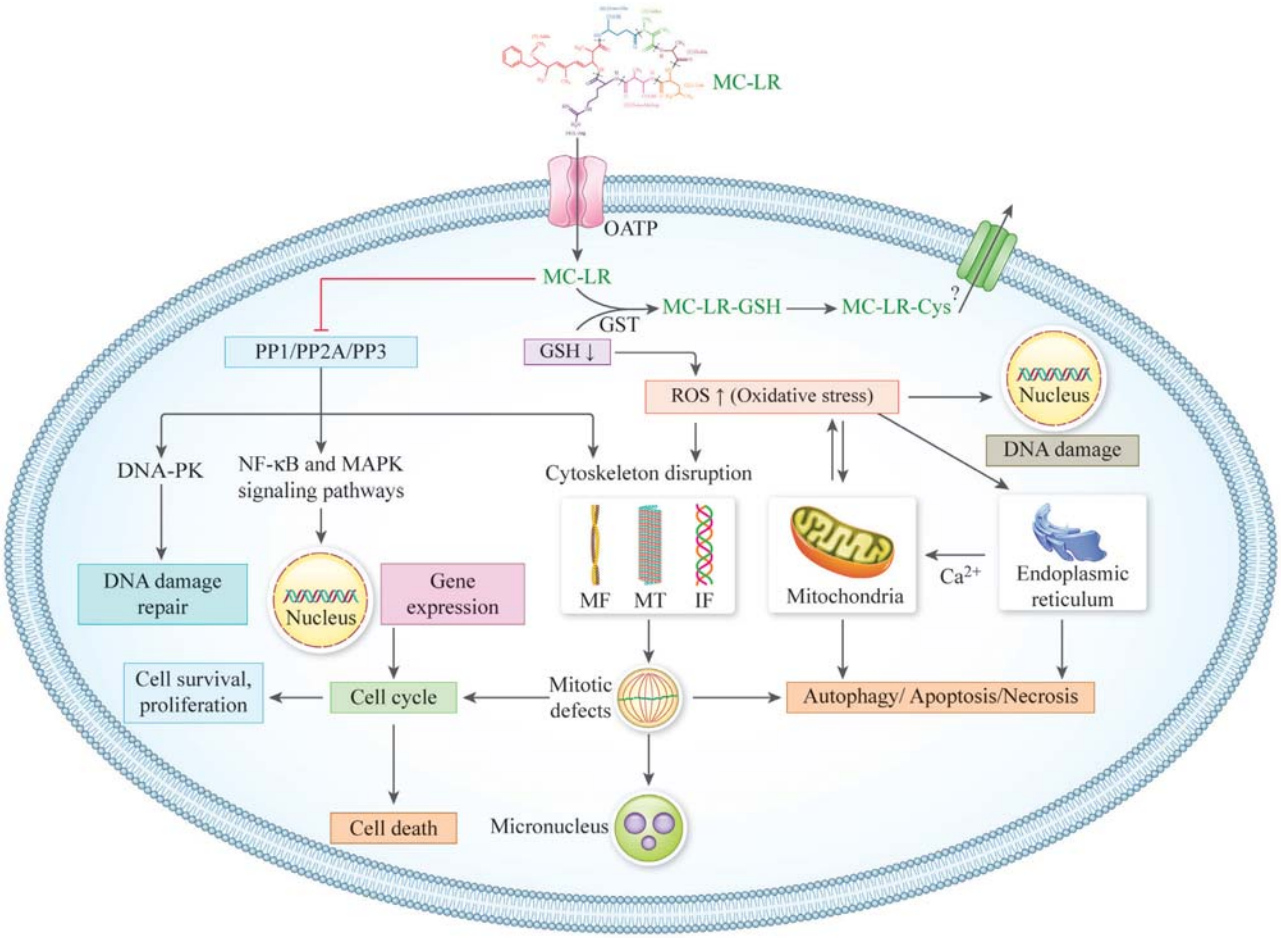

Fig. (3). A schematic review of the mechanisms of microcystin-induced toxicity and cell death. Organic anion transporting polypeptides (OATPs) are responsible for the active transmembrane transport of microcystin (MCs) into the cells. MCs are highly potent and specific inhibitors of eukaryotic protein serine/threonine phosphatases 1 and 2A (PP1 and PP2A), which causes phosphorylation/dephosphorylation imbalance of key control proteins that regulate cytoskeleton organization, DNA damage repair, cellular proliferation and cell death. MCs exposure also induces excessive production of reactive oxygen species (ROS) and oxidative stress, leading to cytoskeletal disruption, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and DNA damage. MCs induce cell apoptosis mediated by the mitochondrial and ROS and ER pathways. Cytoskeleton disruption, cell cycle arrest, and mitosis defects are also involved in MC-induced cell death.

that human hepatoma HepG2 cells are evidently able to tolerate higher concentrations of MC-LR than normal human hepatocytes. In HepG2 cells, MC-LR accumulated to a lesser extent despite a slightly elevated expression of the MC transporter protein, OATP-C. More interestingly, the sensitivity of normal human hepatocytes varied slightly, depending on the donor's age. The viability of human colon adenocarcinoma CaCo-2 cells exposed to 10 μg/mL MC-LR for 24 and 48 h was reduced by about 40%, while that of human astrocytoma IPDDC-A2 and human B-lymphoblastoid NCNC cells was not affected [28]. Intracellular ROS production was increased in CaCo-2 and IPDDC-2A, but not in NCNC cells. Using the comet assay, it was shown that MC-LR, at non-cytotoxic concentrations, induced a time- and dose- dependent increase of DNA damage in CaCo-2 cells, but not significantly in IPDDC-2A and NCNC cells [28]. Humpage and Falconer [29] found that cytokinesis of primary mouse hepatocytes was stimulated and the rate of apoptosis reduced by picomolar (pM) concentrations of MC-LR, whereas at higher (nanomolar, nM) concentrations, cytokinesis appeared to be inhibited and cell death was induced. MC-LR also has a binary effect in Vero-E6 cells (African green monkey-*Cercopithecus aethiops* kidney epithelial cells): the stimulation of cell proliferation at low MC-LR concentrations and the induction of autophagy/ apoptosis at (sub)cytotoxic concentrations of MC-LR [30]. Alverca *et al*. [31] and Menezes *et al*. [32, 33] indicated that Vero-E6 cells respond to MC-LR-induced stress through autophagy, apoptosis or ultimately through necrosis, and the type of response seems to be highly dependent on the strength of the stimulus, *i.e.* the dose and/or the exposure time.

Autophagy is a constitutive physiological process of all cell types and is involved in cellular homeostasis and stress response [32]. This cellular process is mostly described as a mechanism of cell survival that enables cells to undergo temporary starvation or to repair inflicted damages. However, if this is unsuccessful, then the cell death program is activated to eliminate these damaged cells from the organism [34]. Apoptosis, or programmed cell death, is a

complex and highly regulated phenomenon in which cell death is executed through the activation of specific signaling pathways. The morphological changes typical for apoptosis include condensation of cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation, disintegration of mitochondria and formation of apoptotic bodies. In contrast to apoptosis, necrosis has been considered as an accidental mode of cell death for many years and rarely serves the needs of the organism. But there is now mounting evidence that the execution of necrotic cell death is also regulated by a set of signaling pathways [34].

In fact, there is a functional relationship between MC-induced autophagy and apoptosis/necrosis in which, depending on the cellular status, autophagy either avoids cell death (suppressing apoptosis or alleviating cellular stress) or constitutes an alternative cell-death pathway [32]. At lower MC-LR concentrations, autophagy is triggered as a survival mechanism of Vero-E6 cells, in an attempt to eliminate the toxin and/or the MC-LR-induced cellular damages [31-33]. However, the presence of a significant increase in apoptosis/ necrosis at sub-cytotoxic MC-LR concentrations, suggests that in this case, autophagy was ineffective in attenuating the MC-LR-induced cellular damages, triggering a drastic cell death pathway. The results showed that MC-LR exhibited a hormetic dose response relation, characterized by low-dose stimulation of autophagy and a high-dose stimulation of apoptosis/necrosis. The toxic MC effects include an induction of cytoskeletal disruption, cell cycle deregulation, oxidative stress, mitochondrial damage and DNA injury [35]. Many of these cellular events ultimately lead to cell death. MCs were found to induce apoptosis in a variety of cell types, including hepatocytes [36], lymphocytes [37], neuron [38] and kidney epithelial cells [32].

4. PROTEIN PHOSPHATASES

The main mode of action of MCs has been attributed to the highly specific inhibition of serine/threonine protein phosphatases 1 and 2A (PP1 and PP2A), which in turn causes an excessive phosphorylation of proteins [39]. Furthermore, MC-LR and -LA were found to inhibit PP3, order of potency PP2A > PP3 > PP1 [40]. MCs were found to interact with PP catalytic subunits (PP1c and PP2Ac) by a two-step mechanism involving rapid binding and inactivation of the catalytic subunits, followed by a slower covalent interaction (within hours) [41]. Because hydrophobicity may be the initial driving force behind the binding of MC-LR to PP1c, Adda could be responsible for anchoring the cyclic backbone ring into its bound position. The other contributing residues for inhibition, Asp and Glu, have D-oriented, negatively charged carboxyl groups located underneath the saddle which are both oriented to interact with positively charged Arg-96 of PP1c [41, 42]. Finally, the secondary covalent linkage that forms after inhibition is dependent on the modified amino acid Mdha being located at the front and top of the toxin saddle to link covalently to Cys-273 [43]. Similar results were also obtained with PP2A, and interactions between PP2A and MC-LR are strengthened by a covalent linkage between the Sγ atom of Cys269 and the terminal carbon atom of the Mdha side chain [41, 44].

Several studies indicated that MCs modulate PPs activity not only by the direct inhibition of enzyme activity, but also through the regulation of protein expression [45, 46]. PP1/2A inhibition induces the imbalance of protein phosphorylation/dephosphorylation, leading to the dysregulation of numerous signaling pathways such as NF-κB, p38, c-Jun N-terminal kinase (JNK), extra-cellular signal-regulated kinase 1/2 (ERK1/2) of mitogen-activated protein kinase (MAPK) [47, 48]. Douglas *et al*. [49] reported that through the inhibition of PP1/2A, MC-LR may be implicated in the regulation of the activity of DNA-dependent protein kinase (DNA-PK), which is required for DNA double strand break repair by the process of non-homologous end joining (NHEJ). Moreover, the well-known effects of MC-LR on cytoskeletal disruption, metabolic disorder, cell cycle arrest, abnormal cell proliferation and cell death have been related to PP1/PP2A activity and the increased phosphorylation of certain proteins [48, 50].

Recently, several studies demonstrated that low concentrations of MC-LR stimulate, rather than inhibit, PP2A activity, challenging the longstanding role of MCs as PP2A inhibitor [46, 51, 52]. The increased mRNA and protein levels of the PP2A C subunit may explain the increased activity of PP2A [46]. Immunoprecipitation and immunofluorescence assays revealed that the catalytic subunit and a regulatory subunit of PP2A, termed α4, dissociate from inactive complex upon MC-LR exposure, suggesting that the released catalytic subunit regains activity and thereby compensates the activity loss [51]. Ceramide may also mediate the MC-LR-induced up-regulation of PP2A activity and protein level of PP2A regulatory subunits in human embryonic kidney 293 (HEK293) cells [52].

5. OXIDATIVE STRESS

Recent studies showed that excessive production of reactive oxygen species (ROS) and oxidative stress played a significant role in MCs toxicity [53-56]. The main ROS implicated in the tissue injury are superoxide anion ($\bullet O_2^-$) generated in mitochondria, hydrogen peroxide (H_2O_2) produced from O_2 by the action of superoxide dismutase, hydroxyl radical (OH^-) produced from the decomposition of hydroperoxides, and peroxynitrite ($ONOO^-$) generated by the reaction of $\bullet O_2^-$ with nitric oxide (NO) [57]. ROS could be scavenged by the antioxidant defense system, including enzymatic and non-enzymatic mechanisms. Glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) are the important antioxidant enzymes. The non-enzymatic defenses include glutathione (GSH) and vitamin C and E. Thus, oxidative stress is classically defined as "a disturbance in the prooxidant-antioxidant balance in favor of the former", leading to potential damage of basic cellular constituents, lipids, proteins and nucleic acids, including lipid peroxidation (LPO), protein oxidation and DNA strand breaks [58]. However, the major cellular thiol/disulfide systems, including reduced glutathione/oxidized glutathione (GSH/GSSG), thioredoxin-1 ($-SH_2$/-SS-), and cysteine/cystine (Cys/CySS), are not in redox equilibrium and respond differently to chemical toxicants and physiologic stimuli [59]. Therefore,

from a mechanistic standpoint, oxidative stress may be better defined as "a disruption of redox signaling and control".

Guzman and Solter [60] showed that exposure to sub-lethal MC-LR (16, 32, and 48 μg/kg/day body weight via intraperitoneal osmotic pumps for 28 days) led to a dose-dependent increase in malondialdehyde (MDA) concentrations in the livers of male Sprague-Dawley rats. Oxidative lipid metabolism as a result of acute (LD_{50}) MC-LR-induced hepatotoxicity was also observed in male Sprague-Dawley rats [61]. An increase of lipid radicals but a decrease of lipid methylene hydrogen resonances was detected in MC-LR-treated livers. Glutamine, glutamate and lactate levels were also significantly decreased [61]. Swiss albino female mice exposed to 1 LD_{50} MC-LR showed a significant time-dependent induction of HSP-70, an early marker of oxidative stress [54]. Acute exposure to MC-LR also increased or decreased the enzyme activities of GPX, GR, SOD and CAT in the liver and kidney of mice [54, 62] and rats [55, 63, 64]. Moreover, modulation of expression of these antioxidant enzymes was also observed after MCs exposure [54, 62, 64]. MC-LR also markedly enhanced the $\bullet O_2^-$ and H_2O_2 formation in primary cultured rat hepatocytes [65, 66]. Recently, it's shown that MC-LR can bind to CAT to form a complex and lead to conformational and microenvironmental changes of the protein, which may affect its physiological functions and induce oxidative stress [67].

GSH, γ-L-glutamyl-L-cysteinyl-glycine, is the most abundant non-protein thiol in cells and serves several vital functions such as cell growth, proliferation, and apoptosis [68, 69]. GSH is also the most abundant antioxidant and participates in the protection of cells against oxidative stress. GSH also conjugates with electrophiles and thus participates in the metabolism and detoxification of endogenous compounds and xenobiotic toxicants, as a major detoxification agent in cells. It has been shown that MCs bind to GSH, forming conjugates (MC-GSH) via glutathione S-transferase (GST), as the first step in the detoxification process, followed by degradation to the cysteine conjugates (MC-Cys) [70, 71]. The conjugation makes the toxin more soluble in water, supporting the excretion of toxin from the cells and organisms. The use of GSH by the conjugation to MC-LR may trigger an increase in GSH synthesis, possibly by activating the expression of glutamate-cysteine ligase (GCL, formerly γ-glutamyl-cysteine synthetase), and GSH synthetase (GS, also known as GSH synthase) [62, 64]. In fact, GSH is synthesized *de novo* through two ATP-requiring enzymatic steps: formation of γ-glutamyl-cysteine from glutamate and cysteine catalyzed by GCL and formation of GSH from γ-glutamyl-cysteine catalyzed by GSH synthetase, and GCL is the rate-limiting enzyme for GSH synthesis [68, 69]. The initial depletion of GSH and subsequent induction of GSH *de novo* synthesis might suggest a key role of GSH in the detoxification of MC-LR and protection against oxidative stress [55]. There's also evidence that pre-treatment of buthionine-(S,R)-sulfoximine (BSO), a specific GSH synthesis inhibitor, significantly reduced the detoxification of MC-LR and enhanced the level of oxidative stress and hepatotoxicity in SD rats [55, 64, 71].

Ding *et al*. [65] found that rat hepatocytes underwent an apoptotic cell death after exposure to MC-LR in a rather rapid manner. Moreover, a significant rapid increase of ROS level occurred before apoptosis, indicating a critical role of ROS in MC-LR-induced apoptosis. Similarly, in an *in vivo* study, intraperitoneal (i.p.) injection of 60 μg/kg body weight (b.w.) MC-LR prompted large amount of ROS generation in mice liver, up-regulated the expression of Bax and Bid, caused hepatocyte apoptosis as well as liver injury [72]. While pre-treatment with antioxidants, oral administration of vitamin C and E, significantly reduced the generation of ROS and effectively inhibited the MC-LR-induced hepatocyte apoptosis and liver injury. Thus, it's believed that that ROS has an important role in MC-LR-induced hepatocyte apoptosis and liver injury. Our previous study also showed that pre-treatment of BSO enhanced the level of oxidative stress and promoted MC-induced apoptosis [55]. MC-induced oxidative stress and apoptosis were also observed in studies on aquatic animals both *in vivo* [73-75] and *in vitro* [56, 76-78].

6. GENOTOXICITY

In recent years, there is increasing evidence suggesting that MCs induce DNA damage, which means that they are genotoxic [11]. Rao and Bhattacharya [79] reported that i.p. injection of MC-LR at 0.5, 1 and 2 LD_{50} doses (21.5, 43 and 86 μg/kg b.w., respectively) exhibited a dose- and time-dependent DNA damage measured by the fluorimetric analysis of DNA unwinding in liver of male Swiss albino mice, and the DNA damage was partially prevented by GSH. A significant increase of the % tail DNA in Male Fischer F344 rats sub-chronically exposed to MC-YR (i.p injection of 10 μg/kg b.w. every second day for 30 days) was observed in brain (2.5 fold), liver (2.1 fold), kidney medulla (1.9 fold), kidney cortex (1.8 fold) and lung (1.7 fold) cells compared to the control ones, while the DNA from lymphocytes and spleen cells was not affected [80]. Following a single oral administration of MC-LR (2 and 4 mg/kg b.w.), a significant induction of olive tail moment (OTM) and tail DNA in blood cells of female Swiss Albino mice was observed after 3 h but not 24 h after the administration [81]. However, after an acute i.p. injection of MC-LR (10, 25, 40 and 50 μg/kg b.w.), DNA lesions were mainly induced in the liver, but were also reported in the bone marrow, kidney, intestine and colon. Moreover, unscheduled DNA synthesis (UDS) and the comet assays showed no induction of DNA damage in hepatocytes of male Fischer rats following i.v. administration of 12.5, 25 and 50 μg/kg b.w. MC-LR [82]. The discrepancy of results for DNA damages in rodents might be because of the bio-disponibility and kinetics of MC-LR according to the administration route [81, 82].

MC-LR was also found to induce increase in micronucleus (MN) frequency and mutation frequency at the heterozygous thymidine kinase (TK) locus in human lymphoblatoid TK6 cells [83]. Molecular analysis of the TK mutants revealed that MC-LR specifically induced loss of heterozygosity (LOH) at the TK locus, but not point mutations or other small structural changes. Dias *et al*. [84] also reported that MC-LR treatment caused a significant induction of micronuclei in both HepG2 and Vero-E6 cells *in vitro*. Similarly, a positive effect was also observed in reticulocytes of mice i.p. injected with 37.5 μg/kg b.w. MC-LR. Moreover, the

fluorescence in situ hybridization (FISH) analysis of the MN content (HepG2 cells) suggested that MC-LR induces both chromosome breaks and loss [84]. Significantly elevated MN frequency was also observed in bone marrow cells of MC-LR-treated mice [85]. In contrast, Zhan *et al.* [86] demonstrated that oral exposure to MC-LR (1 mg/kg b.w., 0.1 LD_{50}, once every week for 4 weeks) did not increase either MN frequency in the peripheral blood cells or mutant frequencies of the lacZ and cII genes in liver and lungs of the λ/lacZ transgenic mouse. While N-nitrosodiethylamine (DEN) treatment significantly increased MN frequency, the co-exposure of MC-LR did not potentiate its mutagenicity. Similarly, none of the male CBA mice exposed to MC-LR or the cyanobacterial extracts, showed an increased frequency of micro-nucleated polychromatic erythrocytes in the peripheral blood [87]. Also, in an *in vitro* assay, the cyanobacterial extracts did not result in a concomitantly higher frequency of micronucleated binucleated human lymphocytes.

Evidence is accumulating showing that ROS plays an important role in MC-induced DNA damage. Rao and Bhattacharya [79] reported that GSH partially prevented DNA damage in liver of MC-LR treated mice. Žegura *et al*. [88] showed that digestion of DNA with oxidative DNA damage specific enzymes, endonuclease III (Endo III), which catalyzes excision of oxidized pyrimidines, significantly increased DNA strand breaks in MC-LR treated HepG2 cells. Digestion with formamidopyrimidine-DNA glycosylase (Fpg), which catalyzes oxidized purines, predominantly 8-oxoguanine, showed that MC-LR also induced this type of DNA damage. These results suggested that a substantial portion of the MC-LR-induced DNA strand breaks originate from excision of oxidative DNA adducts. Furthermore, the formation of DNA strand breaks and oxidized purines was completely prevented by a superoxide dismutase (SOD) mimic, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL), an iron chelator, deferoxamine (DFO), a precursor of GSH and intracellular ROS scavenger, N-acetyl-L-cysteine (NAC), and partly by hydroxyl radical scavengers dimethylsulphoxide (DMSO) and 1,3-dimethyl-2-thiourea (DMTU), confirming the role of ROS in the induction of DNA damage [88, 89]. Žegura *et al.* [90] showed a correlation between the time course of alterations of intracellular GSH content and the formation and disappearance of MC-LR-induced DNA damage in HepG2 cells. Further studies also indicated that pre-treatment with BSO dramatically increased the susceptibility of HepG2 cells to MC-LR-induced DNA damage, while pre-treatment with NAC almost completely prevented MC-LR-induced DNA damage. Maatouk *et al*. [91] and Bouaïcha *et al*. [92] found that MC-LR treatment induced formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG, 8-OHdG), a DNA oxidative damage marker, in both liver of rats *in vivo* and primary rat hepatocytes *in vitro*. The presence of 8-oxo-dG in DNA will cause misreading and contribute to the oxidative mutational load by inducing G:C to T:A transversion mutations, unless repaired prior to DNA replication. MC-LR also significantly decreased the amount of endogenous formed DNA adducts, termed I compounds, which could play a critical role in cellular transformation and may contribute to carcinogenesis [92]. Nong *et al*. [93] also showed that MC-LR increased DNA strand breaks (indicated by increased tail moment) and 8-OHdG formation in HepG2 cells, and the DNA damage was inhibited by ROS scavengers, catalase (CAT), superoxide dismutase (SOD), and deferoxamine (DFO).

Recently, Wang *et al*. [94] showed that MC-LR caused a dose-dependent increase in the mutation frequency at the CD59 locus and MN formation in human-hamster hybrid (AL) cells, accompanied by an increase of nitric oxide (NO) production. It's known that NO can react with the superoxide anion ($\bullet O_2^-$) to form more reactive and toxic peroxynitrite ($ONOO^-$), which contributes to the hydroxylation and nitration of DNA and mutations. Further analysis revealed that both NO production and mutation frequency induced by MC-LR were suppressed by concurrent treatment with the NO synthase inhibitor N^G-methyl-L-arginine (L-NMMA), confirming the involvement of NO in MC-LR-induced genotoxicity. Moreover, mitochondrial DNA-depleted (ρ^0) AL cells were more sensitive than wild-type cells to MC-LR-induced cytotoxicity, while the CD59 mutant fraction was not affected. Because mitochondrial DNA encodes a nitric oxide synthase (NOS) that is predominantly responsible for the formation of NO, the authors hypothesized that the genotoxic effects of MC-LR were mediated by NO and mitochondria [94].

Chen *et al*. [95] found that MC-LR exposure induced mitochondrial DNA (mtDNA) damage in rat testis. In fact, mtDNA was more sensitive than nuclear DNA (nDNA) to oxidative damage because the mitochondria are in close proximity to the free radical-producing electron transport chain (ETC). Increased 8-OHdG levels of DNA, impairment of integrity of mtDNA and nuclear DNA (nDNA), and altered mtDNA content were also observed in the liver of male C57BL/6 mice received long-term and persistent oral exposure to MC-LR (1, 5, 10, 20 and 40 μg/L for 12 months) [96]. Notably, MC-LR exposure changed the expression of mitochondrial and nuclear genes that are critical for regulating mtDNA replication and repairing oxidized DNA.

Douglas *et al*. [49] reported that MC-LR may be implicated in the regulation of the activity of DNA-dependent protein kinase (DNA-PK), which is required for DNA double strand break repair by the process of non-homologous end joining (NHEJ). Subsequently, Lankoff *et al*. [97, 98] found that MC-LR inhibited the repair of γ radiation-induced damage and enhanced frequencies of chromosomal aberrations including dicentric chromosomes in human lymphocytes. Moreover, pre-treatment with MC-LR resulted in reduced numbers of γ-H2AX foci in irradiated cells. The impact of MC-LR on DNA-PK was then confirmed by the strong DNA repair inhibitory effect observed in the human glioblastoma MO59K (DNA-PKcs-proficient) cells but not in the MO59J (DNA-PKcs-deficient) cells irradiated with X-rays. Lankoff *et al.* [99] also demonstrated that MC-LR targeted the nucleotide excision repair (NER) mechanisms by interference with the incision/excision phase as well as the rejoining phase of NER and led to an increased level of ultraviolet (UV) radiation-induced DNA damage in CHO-K1 cells. Hence, it is highly likely that the interference of DNA

repair process may be one of the mechanisms responsible for the MC-promoted cancer development.

7. CYTOSKELETON DISRUPTION

The cytoskeleton is a well-organized network of intracellular filaments and consists of three major components: microfilaments (MFs), microtubules (MTs) and intermediate filaments (IFs) [50]. These elements not only provide cellular architecture to maintain cell shape, cell division, migration, adhesion and contraction/relaxation, but also play a central role in signal transduction. Both *in vivo* and *in vitro* studies have shown that exposure of human, rat and fish hepatocytes to MC-LR induce alteration, aggregation, breakdown, reorganization and collapse of the cytoskeleton, loss of intercellular contacts and consequently disruption of cellular architecture [75, 100-102]. Cytoskeletal alterations induced by MC-LR were also reflected in plants, *Vicia faba* [103] and *Ceratophyllum demersum* [104].

The inhibited PP1 and PP2A by MCs were shown to induce the hyperphosphorylation of the liver IF proteins, keratins 8 and 18, associated with morphological changes in rat hepatocytes [105, 106]. Similarly, hyperphosphorylation of MF proteins (ezrin, vasodilatorstimulated phosphoprotein and cofilin), MT proteins (tau) and IF proteins (keratin8/18 and vimentin) were also observed in human normal liver HL7702 cells [107-109], liver cancer SMMC-7721 cells [110] and neuroendocrine PC12 cells [111] exposed to MC-LR, regulated through PP2A and MAPK pathway. Besides, MC-LR also increased the phosphorylation of the actin-associated protein HSP27 in MC-LR-treated HL7702 [47], SMMC-7721 [110], and PC12 cells [111].

In addition to phosphorylation regulation, Ding *et al*. [66, 112] revealed that ROS formation and oxidative stress also played a crucial role in MC-induced disruption of cytoskeleton organization and consequent hepatotoxicity. Pre-treatment with N-acetyl-cysteine (NAC), 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL) or desferoxamine (DFO) significantly decreased the MC-induced cytoskeleton changes in primary rat hepatocytes. Meng *et al*. [113] demonstrated that MC-LR induced time- and concentration-dependent ROS generation, p38-MAPK activation and tau hyperphosphorylation in PC12 cells. However, all of these effects were significantly attenuated by the antioxidants, NAC and vitamin C, suggesting that ROS generation triggered by MC-LR is a key intracellular event that contributes to an induction of p38-MAPK activation and tau phosphorylation. Pre-injection of the antioxidant NAC also provided significant protection to the cytoskeleton system in the liver of common carp (*Cyprinus carpio* L.) exposed to MC-LR, however buthionine sulfoximine (BSO) exacerbated cytoskeletal destruction [75].

Many transcriptomic [114-116] and proteomic studies [45, 117-128] have indicated that MCs (-LR, -RR) exposure altered the expression of massive cytoskeletal and cytoskeleton-associated proteins both *in vivo* and *in vitro*. Chen *et al*. [95] found that the homeostasis of the expression of cytoskeletal genes in rat testis was significantly affected after exposure to MC-LR. In an *in vitro* study, Huang *et al*. [56] reported that MC-LR caused aggregation and collapse of MFs and MTs in *Ctenopharyngodon idellus* kidney (CIK) cells, and even loss of some cytoskeleton structure. Moreover, transcriptional changes of cytoskeletal genes (β-actin, lc3a, and keratin) were also determined. The authors hypothesized that the morphological damages of cytoskeleton induced by MC-LR could be either the cause or the result of altered expression of cytoskeletal genes.

Although there's no evidence of direct association between cytoskeleton disruption and apoptosis in MCs toxicity, several studies suggested that cytoskeleton disruption may be a key event in MC-induced apoptosis [56, 129, 130]. In fact, in addition to mediating the morphological modifications of the apoptotic cell, several proteins of the cytoskeleton such as actin and keratins are also involved in the regulation of apoptotic signaling [131]. The apoptotic shrinking of cells caused by MC-LR can be at least partially explained by the shortening of MFs and by the degradation of the MTs known as the major cytoskeletal components of eukaryotic cells [129].

8. MITOCHONDRIA DYSFUNCTION

Mitochondria are known to be vulnerable targets of various toxins because of their important role in maintaining cellular structures and functions [53]. Ding *et al*. [65] found that MC-LR-induced reactive oxygen species (ROS) formation led to onset of mitochondria permeability transition (MPT) and loss of mitochondrial membrane potential (MMP). Deferoxamine (DFO), an iron chelator, prevented the increase of ROS production, delayed the onset of MPT and subsequent cell death. In addition, a specific MPT inhibitor, cyclosporin A (CsA), blocked the MC-LR-induced onset of MPT, mitochondrial depolarization and cell death. Mitochondrial calcium (Ca^{2+}) also played an important role in the onset of MPT and cell death in MC-LR-treated rat hepatocytes [132, 133]. Zhang *et al*. [76, 77] demonstrated that MC-LR- and MC-RR-induced apoptosis was associated with a massive intracellular Ca^{2+} elevation, resulting in O_2^- generation, MMP disruption and ATP depletion. Huang *et al*. [134] also revealed that the mechanism of MC-RR-induced apoptosis signaling pathways in tobacco BY-2 cells involved not only the excess generation of ROS and oxidative stress, but also the opening of MPT pore inducing loss of MMP.

There is substantial evidence indicating that mitochondria work as a central executioner in the apoptotic signaling pathway, and MPT is implicated as a critical, rate-limiting event [65]. The main mechanism of outer mitochondrial membrane (OMM) permeabilization in apoptosis involves the pro-apoptotic members of the Bcl-2 family of proteins [135]. Bcl-2 family consists of both pro-survival (Bcl-2, Bcl-xL, Bcl-w, etc.) and pro-apoptotic members (Bax, Bak, Bik, Bim, etc.). MCs were found to significantly induce Bax but suppress Bcl-2, leading to the release of cytochrome c (cyt c) and caspase cascade and subsequent induction of apoptosis, in various cell types of fish, frog, mouse, rat and human [136-143].

Mikhailov *et al*. [144] showed that MC-LR can bind to β subunit of ATP-synthase, and the binding may be a trigger of mitochondrial apoptotic signaling through perturbation of

mitochondrial functions, with possible leakage of cytochrome c. Human liver mitochondrial aldehyde dehydrogenase 2 (ALDH2) is also a potential target of MC-LR, and ALDH2 dysfunction may lead to aldehyde-induced ROS generation and, in turn, apoptosis [145].

La-Salete *et al*. [146] examined the effect of MC-LR on mitochondrial respiratory chain and oxidative phosphorylation system of rat kidney isolated mitochondria. MC-LR decreased both state 3 and carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP)-uncoupled respiration. The MMP was strongly depressed by MC-LR in a concentration-dependent manner, pointing to an uncoupling effect. Further study showed that the transmembrane decrease was a consequence of a strong inhibitory effect on redox complexes, including succinate dehydrogenase, succinate cytochrome c reductase, cytochrome c oxidase, ATP synthase and ATPase. MC-LR induced the opening of the MPT pore, as evidenced by mitochondrial swelling in isosmotic sucrose medium. Mitochondrial swelling in the presence of Ca^{2+} was inhibited by cyclosporin A (CsA), catalase (CAT), and dithiothreitol, indicating the participation of mitochondrial generated ROS in this process [146].

After exposure to MC-LR, transcription of 8 mitochondrial genes related to electron transport chain (ETC) and the oxidative phosphorylation (OXPHOS) system, including cytochrome c oxidase (-I, -II, -III), NADH dehydrogenase (-1, -3, -6) and ATP synthase (-6, -8), were significantly elevated in testes of rats [95]. Swelling mitochondria were also observed. Since ROS formation is intrinsically related with mitochondrial energy metabolism and mitochondrial ETC is considered as a major intracellular source of ROS, mainly at the level of the complex I and III, we think that MC-induced mitochondrial dysfunction is responsible for promoted ROS formation and oxidative stress. Mitochondrial ETC also played a key role in mediating MC-RR induced apoptosis in tobacco BY-2 cells through an increased mitochondrial production of ROS [147].

9. ENDOPLASMIC RETICULUM STRESS

Recently, another intrinsic apoptotic pathway triggered by endoplasmic reticulum stress (ERS) in addition to the mitochondrial pathway has been reported in MCs toxicity [32, 137]. MCs induced swelling and vacuolization of endoplasmic reticulum (ER) in Vero-E6 cells [31] and tobacco BY-2 cells [148]. ER is one of the largest cellular organelles and is responsible for the regulation of protein translocation, protein folding, protein post-translational modifications and maintenance of calcium (Ca^{2+}) homeostasis [149]. Correctly folded proteins can be transported out of the ER, to the Golgi apparatus and ultimately to vesicles for secretion or display on the plasma surface. However, numerous perturbations including xenobiotics exposure, oxidative stress, hypoxia, aberrant Ca^{2+} regulation, and energy balance disruption can disturb ER homeostasis, leading to the accumulation of unfolded or misfolded proteins inside the ER, a cellular condition referred to as ER stress [149, 150]. To combat ERS, cells have evolved a highly conserved adaptive stress response, *i.e.* the unfolded protein response (UPR), including up-regulation of molecular chaperones (GRP78 and GRP94), enhancement of ER protein folding capacity, decrease in protein synthesis to diminish ER load or increased activity of ER-associated protein degradation (ERAD) pathways for the removal of misfolded proteins [32, 150]. UPR signaling affords the cell a "window of opportunity" for stress resolution and thus temperate ERS can relieve cellular dysfunction and increase the possibility for cell survival; however, serious and/or prolonged ERS leads to apoptosis, which is mediated by transcriptional induction of C/EBP homologous protein (CHOP) and/or by a caspase-12-dependent pathway [150, 151]. ER-targeted stress can also activate Ca^{2+} efflux from the ER; this Ca^{2+} is then redistributed to the mitochondria and leads to apoptosis [148].

Xing *et al*. [137] found that MC-LR significantly up-regulated CHOP protein and induced apoptosis in human amniotic FL cells. It has been reported that over-expression of CHOP can lead to translocation of Bax protein from the cytosol to the mitochondria and decrease in Bcl-2 protein and finally trigger the mitochondrial pathway. MC-LR significantly improved mRNA and protein expression of CHOP and cleaved caspase-12 in mouse liver, whereas it inhibited expression of CHOP and caspase-12 in kidney [151]. The expression levels of GRP78, which may stabilize protein folding under ERS and protect cells from apoptosis, were significantly increased in MC-RR-treated mouse liver, but no obvious alteration was found in CHOP expression [152]. These results indicated that MC-RR treatment had induced ERS, but it was still within the capability of the homeostasis of ER. MC-LR also induced a concentration-dependent decrease of GRP94 expression in HepG2 cells, suggesting the involvement of the ERS in apoptosis [32]. Increased CHOP and decreased GRP78 were also observed in the testes of male frog exposed to MC-LR [153]. Christen *et al*. [154] demonstrated that exposure of human hepatoma (Huh7) cells to MC-LR leads to the activation of ER stress response and all three UPR signaling pathways, including IRE1, PERK and ATF-6.

10. CELL CYCLE REGULATION

Although toxicants may initiate cell damage or stress, the cellular proteins that are involved in control of cell cycle and apoptosis are the final arbiters of cell fate [155]. Apoptosis and proliferation are intimately coupled and are linked by cell cycle regulators and apoptotic stimuli that affect both processes [156]. MCs promoted cell survival and growth at lower doses, while induced apoptosis/cell death at higher doses in liver cells [29, 157], lymphocytes [158], kidney cells [30-33, 56], HeLa cells [139]. It is accepted that both survival and death signals could be activated by MCs, and the cell responded to the stronger signals, so the outcome of the MCs on the cells depends on concentration of the toxin as well as duration of exposure [139, 142, 159, 160].

Eukaryotic cell cycle, a highly conserved and ordered set of events, has checkpoints between its four phases G_0/G_1, S, G_2 and M, to regulate accurate cell division and growth and prevent the replication of damaged DNA and cellular damage [139]. The major control sites include DNA damage

checkpoints (G_1/S, intra-S, G_2/M) and spindle checkpoints. Checkpoint signaling may induce apoptosis if cellular damage cannot be properly repaired. Defects in cell cycle checkpoints can result in gene mutations, chromosome damage and aneuploidy, all of which can contribute to tumorigenesis [155]. Lankoff er al. [161] indicated that MC-LR effectively deregulated the cell cycle of CHO-K1 cells by damaging the mitotic spindle apparatus and induced both apoptosis and necrosis. Giemsa staining of cells treated with MC-LR revealed a dose- and time-dependent increase of mitotic indices, accumulation of abnormal G_2/M figures with hypercondensed chromosomes, abnormal anaphases with defective chromosome separation, and polyploid cells. The majority of the mitotic cells (metaphase, anaphase and telophase cells) showed monopolar and multipolar mitotic spindles (multiple asters). Abnormal condensation of microtubules (microtubule bundles) was present in interphase cells. MC-LR induces apoptosis and necrosis in a dose- and time-dependent manner and the frequency of dead cells is positively correlated with the frequency of polyploid cells. Gácsi *et al*. [129] also demonstrated that MC-LR caused cytoskeleton damage, reduced the number of mitotic cells, prevented chromosome formation and induced apoptosis and necrosis in CHO-K1 cells. Recent studies showed that relative lower doses of MCs could induce G_1/S progression and cell proliferation while higher doses of MCs lead to G_1/S arrest and apoptosis [30, 56, 139, 162, 163]. Several signaling pathways such as Nrf2, ERK1/2 and NF-κB may be involved in the deregulation of cell cycle induced by MCs. In *Vicia faba*, MC-LR was also shown to induce histone H3 hyperphosphorylation and microtubule anomaly, both of which would lead to incomplete sister chromatid segregation, micronucleus formation and alteration of metaphase-anaphase transition [103].

11. CONCLUSION

Microcystins (MCs) are a group of cyclic heptapeptide toxins produced by several genera of cyanobacteria. In this review, we have focused the intracellular biochemical and molecular mechanisms proven to be responsible for toxicity and cell death, mainly apoptosis, induced by MCs (Fig. **3**). MCs are known to be transported into the cells through cell membranes by organic anion transporting polypeptides (OATPs). Inhibition of protein phosphatases (PPs), which leads to the hyperphosphorylation of cellular proteins, is a possible mode of action of MCs. MCs exposure can also induce excessive formation of reactive oxygen species (ROS) that culminate in oxidative damage. It has been suggested that either of the above mechanisms could induce cytoskeletal disruption. There is increasing evidence suggesting that MCs induce DNA damage, which means that they are genotoxic. Mitochondria appear to serve as central mediators in the apoptosis induced by MCs. Another intrinsic apoptotic pathway triggered by endoplasmic reticulum stress (ERS) has also been reported in MCs toxicity. Deregulation of cell cycle and mitosis is also responsible for MC-induced cell death. Future studies are needed to clarify the whole cascade of events involved in the development of MC-induced toxicity and cell death, and the mechanisms of their regulation, as well as the cooperative/interactive effects and cross-talk of different molecular and cellular targets.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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