Chapter

Mechanisms of Cyanotoxin Toxicity—Carcinogenicity, Anticancer Potential, and Clinical Toxicology

Deyana Georgieva Vankova, Milena Gincheva Pasheva, Yoana Dimitrova Kiselova-Kaneva, Dobri Lazarov Ivanov and Diana Georgieva Ivanova

Abstract

Cyanoprokaryotes are distributed worldwide and they produce various bioactive compounds, including cyanotoxins. The major route of human exposure to cyanotoxins is the oral intake by using contaminated drinking water, by incidental intake of contaminated water during recreational and professional activities, and by consuming contaminated food or dietary supplements prepared from cyanobacteria. The prolonged chronic exposure to low concentrations of cyanotoxins provokes cell damage and may increase the risk for cancer development. Due to the variety of cyanotoxin chemical structures, different mechanisms of their toxic effects are known. At the same time, some of the natural compounds produced by cyanoprokaryotes have anticancer potential and are promising sources for the development of novel drugs. This chapter is dedicated to the target mechanisms behind the effects of the widely distributed cyanotoxins with an impact on human health, microcystins, nodularins, and cylindrospermopsin.

Keywords: cyanotoxins, microcystins, nodularins, cylindrospermopsin, toxicity mechanisms

1. Introduction

Cyanoprokaryotes are Gram-negative photosynthetic algae considered to have arisen approximately 3.5 billion years ago [1]. In nature, they are found as single cell species or as colonies rapidly growing in fresh water, all types of aquatic ecosystems, and terrestrial habitats. Cyanobacteria are photosynthetic organisms, and as such, they are considered primary first-level consumers in the food chains in water ecosystems. Blue-green algae play an important role in carbon and nitrogen balance in the biosphere [2]. They produce a high number of bioactive molecules, and certain species produce cyanotoxins that contribute as defense mechanisms against different ambient stress factors [3]. The growth of cyanobacteria at high blooming densities increases in expansion and frequency following anthropogenic activities and climatic change, globalization, and increasing commodity exchanges [4]. This, in turn, raises morbidity and death rates of wild and domestic animals [5, 6] and brings some risk to human health.

More than 90 microcystin isoforms, that are cyclic peptide cyanotoxins, have been described. The microcystin-leucine arginine (MC-LR) is known as the most toxic and the most abundant variant of microcystins [7]. Several authors have reported that MC-LR has been considered as the most widely spread microcystin in Portuguese waters [8]. However, Rodrigues et al. report similar results for microcystin MC-RR (a MC variant with the amino acid arginine in positions 2 and 4) [9]. MC-RR is the major toxin variant found in the rivers, lakes, and reservoirs in China [10] reaching concentrations of up to 93.5% in the cells of cyanoprokaryotes [11], thus associated with the contamination produced by intensive use of water sources and fast economic development [12]. In Bulgaria, a country rich in water reservoirs and natural water bodies, many cases of cyanoprokaryote blooms have been reported. Surveys conducted for a period of 15 years (2000–2015) in 120 Bulgarian water basins have recorded cyanobacteria blooms in 14 water bodies and have identified 16 cyanotoxins (microcystins LR, LA, RR, YR, nodularins, and saxitoxins) [13].

Cyanotoxins have various chemical structures; thus, their toxic effects are due to different mechanisms. Cyanotoxins are classified into three major groups according to their chemical structure: alkaloids (cylindrospermopsin, saxitoxin, lyngbyatoxin-a, and aplysiatoxin,), cyclic peptides (microcystins, MCs, and nodularins—NODs), and lipopolysaccharides [14]. Poisoning of humans with cyanotoxins is possible through various pathways, mainly by the consumption of contaminated food (vegetables, fish, seafood, and livestock), as well by bathing and recreational activities with contaminated water [15]. Different studies have reported high accumulation of cylindrospermopsin (CYN) in fish (up to 2.7 ng/g) [16], in mussels (up to 2.52 mg/g) [17], and in lettuce (up to 8.029 μg/kg) [18].

Along with the reports about the toxicity of cyanobacteria metabolites, there are studies describing their anticancer properties, hence, viewing them from a new perspective as novel potential sources for anticancer drug development [19]. However, to identify possible drug targets, the science about the mechanisms of the toxicity needs to be extracted out of the numerous scientific reports and review studies on cyanobacteria blooms, case studies and investigations on the effects of cyanotoxins described by different authors.

This review addresses the target mechanisms behind the effects of widely distributed groups of cyanotoxins with an impact on human health, the cyclic peptides microcystins and nodularins, and the alkaloid cylindrospermopsin.

Data collection was performed through keyword research, namely cyanotoxins, microcystins, nodularins, cylindrospermopsin, cyanotoxins/microcystins/nodularins/cylindrospermopsin molecular mechanisms/carcinogenicity/anticancer potential/clinical toxicology/poisoning incidence/clinical toxicology. ScienceDirect and PubMed databases were screened for the above-mentioned key words. More than 100 papers were examined and bibliography includes references dating back to 1878.

2. Water blooms, human and animal health

A great number of studies about cyanotoxins discuss their toxicity from different points of view. Clinical intoxication cases and epidemiological studies on reported cases of exposure to cyanobacteria and their toxins are described [20]. There are reports on acute poisonings of animals and humans due to exposure to cyanotoxins [21, 22]. Chronic intake of contaminated water; aerosolization, including respirable bioaerosols; consumption of contaminated seaborne food [23]; or even intake of dietary supplements containing blue-green algae are investigated and reported [24].

Observational studies on the correlation between clinical symptoms and contact with blooming water have been recorded throughout the centuries. The earliest report on such poisoning dates back 1000 years ago in China when green-colored river water consumption caused mortality in General Zhu Ge-Ling's troops, according to data reviewed on the website of National Toxicology Program, Department of Health and Human Services of USA [25]. Later in 1878, cyanotoxin poisoning was suspected in Australia [26]. In several US states, gastroenteritis has been suspected to be related to water blooms [27]. In China, primary liver cancer has been attributed to cyanotoxin-contaminated drinking water [28]. In a profound recent review, Svircev et al. [20] identified 42 publications that describe 33 cases of cyanotoxin poisoning in 11 countries—Australia, Brazil, Canada, China, Namibia, Portugal, Serbia, Sri Lanka, Sweden, the UK, and the USA for the period between 1960 and 2016. Although there is no definitive general conclusion in the epidemiological literature, it identifies a possible link between microcystins and cancer and other human health issues [20]. Wood [21] presents an informative table summarizing reports about acute animal and human poisonings attributed to exposure to cyanotoxins since 1800; there is an estimate of the number of affected animals and individuals in incidents of mortality and morbidity from 1900 onwards. The author identified 115 human incidents of cyanotoxin intoxications reported until the year 2010, mostly seen in the United States and Canada, followed by Europe [21]. Taking into account the great variety of cyanobacteria and their overall environmental distribution in fresh and brackish waters and the fact that more than 90 different types of cyanotoxins are produced by the blue-green algae, the various routes of cyanotoxin poisoning, as well as the variety of clinical manifestations encountered, we may expect that the above numbers are quite underestimated. In Varna region, Bulgaria, no evidences to related cases of acute poisoning to cyanotoxins have been documented according to local database [118]. Furthermore, it is not always easy to derive information about countries classified by Woods as "the rest of world," especially when information dated back centuries ago. Sometimes, data are published in the gray literature and in the local language; data available are not supported by adequate scientific information. Often clinical evidences of inflammatory response or allergic reactions are misleading for being common symptoms for other types of intoxications as well; such clinical cases remain in the group of *idiopathic* intoxications and are not reported as cases of cyanoprokaryota poisonings. Cyanobacteria content in the total mass of phytoplankton in different waters and sampling periods may vary up to 100%. Usually, in algae blooms, one species predominates and it releases various cytotoxins to the water. Some toxins have been detected even after the end of algae blooms, when cyanoprokaryota species are already in negligible concentrations [13].

Few reports describe correlation between defined clinical symptoms and/or laboratory findings and a reported contact and/or consumption of contaminated water. The most severe human intoxication with cyanotoxins occurred in Brazil in 1996, where 100 of 131 dialysis patients developed acute hepatic failure due to cyanotoxin contamination of the dialysis water applied. The death of 52 of them has been confirmed to be due to the presence on cyanotoxins in treatment water provided from a local water treatment plant [29, 30]. Another incidence, again in Brazil, associates 2000 cases of gastroenteritis and 88 deaths with blooms [31]. *Microcystis aeruginosa*–contaminated water caused pneumonia in two patients in Staffordshire, England [32]. In Australia, 140 children and 10 adults have experienced liver and kidney problems. Cylindrospermopsin is reportedly the etiological agent [33]. Giannuzzi et al. [34] report a case from Argentina in 2007. Microcystin-LR is detected in water samples, where the patient has been immersed before experiencing acute clinical symptoms. In addition, laboratory examination identified increase of markers for liver injury (ALT, AST, and GGT).

3. Cyclic peptides—toxicity and biotransformations

The cyclic pentapeptide nodularins and cyclic heptapeptide microcystins are the most widespread cyanotoxins in water blooms. MCs are produced by different cyanobacterial species (*Microcystis, Oscillatoria, Aphanocapsa, Cyanobium, Arthrospira, Limnothrix, Phormidium, Hapalosiphon, Anabaenopsis, Nostoc, and Synechocystis*). It is known that nodularins are produced only by cyanobacteria from the genus *Nodularia* (*Nodularia spumigena)* [35]. Various investigations reveal approx. 100 known variants of MCs up-to-date with the most toxic and widely distributed MC being MC-LR [36]. The maximum concentration of MC-LR is up to 1 μg/L in drinking water. This is the conditional guideline value adopted by the World Health Organization (WHO). The value is based on tolerated daily intake (TDI) 0.04 μg/kg body weight [37].

Most of the microcystins have hydrophilic structure; thus, their cell uptake should be facilitated by transporting systems, such as the organic anion transporting polypeptides (OATPs). Many OATPs are expressed in a tissue-specific way, whereas others are expressed ubiquitously [38]. A selective uptake of MCs by the cells, depending on the organ type and on the expression of different OATPs, is established in intestines, liver, muscle, and brain cells of three different catfish species [39]; the highest contents are found in liver, gonads, stomach, heart, and kidneys in Wistar rats [40]. The fact that MC accumulation is primarily in the liver is explained by the amount of OATPs present in this organ, which is why MCs are considered as hepatotoxins. More specifically, the MC-LR has been determined as a substrate for OATP1A2, OATP1B1, and OATP1B3. MCs are established to require active transport for human cell uptake, and the high expression of these OATP1B1 and OATP1B3 transporters in the liver accounts for their selective liver toxicity [41, 42]. Researches reveal that OATP1B1 and OATP1B3 expression is detected in cell lines originating from liver, colon, and pancreatic tumors [43], as well as in hepatocellular carcinoma [44]. These results account for microcystin toxicity to be examined mainly in hepatocytes *in viv*o and in cultured hepatic cells *in vitro*.

The liver of the fish species *O. bonariensis*, collected from a shallow lake in Argentina, contained 10 times higher MC-RR levels than the muscles, reports a study [45]. Another study, on mice, elucidates that 9% of the MC-LR toxin fraction is filtrated in the kidneys and eliminated through urine, thus making kidneys a possible object for MC-LR toxicity as well [46]. Pflugmacher et al. [47] determine that the first stage in the detoxification of MCs is the formation of a glutathione conjugate of MC (MC-LR-GSH) in hepatocytes, identified earlier in mice and in rat livers [48]. MC-LR-GSH is then further metabolized to a cysteine conjugate (MC-LR-Cys) for excretion via urine [49], via feces as well, as free MCs or their metabolites [50].

Chronic exposure to sublethal doses of MCs could lead to induction of oxidative stress, necrotic cell death, and liver neoplasia in animals [51] and a possible reason for that could be the depletion of GSH cell stores. A study demonstrates the bioaccumulation of microcystin-LR (MC-LR) in mammals [52]. A pig experimental model has been used because of its liver, kidney, and gastrointestinal tract function similarities to humans, as well as similarities to humans' metabolic rates [53]. Although MC-LR is not found in the serum of treated animals, free MC-LR is detected in the large intestine and kidneys, in liver as well, where the MC-LR-glutathione conjugate is in high quantity, approximately 1.1% of the applied MC-LR dose. The chemical structures of unabsorbed MCs are not modified, or few changes occur. MCs are transported to the intestine and further are excreted by feces. The other route is absorption and subsequent conjugation in the liver

and rapid excretion of this compound in the bile [54]. MC-LR and its derivatives (MC-LR-GSH and MC-LR-Cys) may also enter the enterohepatic circulation and, being reabsorbed into the blood stream, may reach the brain, heart, lungs, and even testicles [55].

Animal studies mark the possible MC-LR accumulation in human hepatic tissue exposed chronically to high doses of cyanotoxins as well. Present-day study analyses daily exposure to MCs and their effects on human health; authors identify the presence of MCs in anglers' serum, most likely resulting from exposure to ingested MC from consumed fish [56]. Serum enzymes aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline phosphatase, which are biomarkers of hepatic function, are elevated, pointing to liver damage in the fishermen. The smaller ring structure of NODs is taken up by the liver cells more easily than MC-LR, thus probably resulting in stronger hepatocellular effects [57]. Another research provides evidence that MC-LR can be transported across the blood-brain barrier in humans [41].

3.1 Cyclic peptides—molecular toxicity mechanism in relation to carcinogenicity

Different mechanisms of cytotoxicity are observed among MC variants and nodularins in a range of *in vitro* cell culture studies on cell viability and the ability to cause apoptosis or necrosis in varying concentrations applied to different cell types. Fastner et al. [58] explicates that primary rat hepatocytes are less susceptible to MC-RR (EC_{50} 1500–4300 nM) compared to MC-LR (EC_{50} 60–200 nM). Independently of the cell culture type (primary or transfected hepatocytes), MC-RR is always less cytotoxic than MC-LR [59]. Gacsi et al. [60] have studied the effects of MC-LR on cultured Chinese hamster ovary cells (CHO-K1) in order to detect cell viability and to determine if nonviable cells go through necrosis or apoptosis. The study demonstrates that low dose of MC-LR (<10 μM) after 24 h exposure does not induce apoptosis in the cell line. The application of higher MC-LR concentrations (⩾20 μM) shows induced apoptosis in a concentration-dependent manner. The shrinkage of apoptotic cells is linked to the shortening and loss of actin filaments and microtubule depolymerization. No necrosis is observed over the concentration range tested. Piyathilaka et al. [61] evaluates the MC-LR cytotoxic and apoptotic effects on different human kidney cell lines—normal embryotic (HEK-293) and adenocarcinoma cell line (ACHN). The MTT and sulforhodamine B (SRB) cell viability assays establish that MC-LR is more cytotoxic to embryonic kidney cells compared to kidney adenocarcinoma cells after treatment with MC-LR for 24 h. In addition, morphological studies also reveal higher MC-LR toxicity to kidney cancer cells than to normal kidney cells. MC-LR does not promote cell division of human kidney adenocarcinoma cells, indicating that it cannot be a promoter of kidney cancer [61].

NODs and MCs are among the most common natural cyanotoxins. Their toxicity is mainly due to the ability to inhibit the eukaryotic protein serine/threonine phosphatase families 1 and 2A (PP1 and PP2A), which are essential for many signal transduction pathways of eukaryotic cells. This inhibition is linked to protein hyperphosphorylation, thus leading to modification of cytoskeleton and disturbances of many cellular processes: loss of cell-cell adhesion at the desmosomes [62], disruption of actin filaments [63], and altered cell signaling pathways, for example MAPKs signaling pathways that regulate cellular proliferation [55]. As potent inhibitors of protein serine/threonine phosphatase, MCs and NODs have a profound effect on cell signaling leading to the affected cell's death. Both MC-LR and NOD have inhibitory effect on protein phosphatases, independently that MC-LR binds covalently to them and NOD does not [64].

Cell signaling pathways involving MAPKs regulate cellular proliferation through phosphorylation cascades. Several types of phosphatases including the protein serine/threonine phosphatases 2A regulate the Ras-Raf-MEK-ERK cascade (PP2A). The phosphatase PP2A inhibits these pathways by dephosphorylation. The activated (phosphorylated) forms of the transcription factor ERK1/2 are translocated to the cell nucleus, thus leading to the transcription of certain proto-oncogenes [65]. Junttila et al. [66] speculate that by inhibiting PP2A, MC-LR could deregulate the ERK1/2 pathway, thus promoting cell proliferation and tumorigenesis.

NODs and MCs also play a role as potential oxidants, which could induce reactive oxygen species production, hence causing cell oxidative stress damages [67]. Many studies demonstrate that oxidative stress is involved in the liver cell toxicity due to MCs [68] and NODs [67]. Increased production of reactive oxygen species (ROS) and lipid peroxides in mouse liver because of treatment with NODs is observed [69].

MCs may increase the production of ROS by depletion of GSH due to a high rate of conjugation [8, 67]. These observations are confirmed in zebrafish [70], where MC administration leads to lipid peroxidation and a change in the antioxidant enzyme activity [71]. A study explicates MC-RR influence on gene expression of nuclear factor—erythroid 2 related factor 2 (Nrf2), a master regulator of inducible antioxidant responses, in human hepatocytes, causing mitochondria dysfunction [72]. The transcription factor Nrf2 has been identified as a key factor in the cell protection from oxidative stress and electrophilic insults [73, 74]. Many of Nrf2 target genes play essential role in maintaining cellular antioxidant responses and xenobiotic metabolism. Its constitutive activation may contribute to a malignant phenotype [75], and its elevated expression and activity have been observed in different cancer cells [76]. Nrf2 promotes the survival of tumor cells under a deleterious environment and elevates resistance to antitumor drugs [77]. These observations suggest that Nrf2 plays contrasting roles in different tumorigenesis stages and is subject to MCs' toxicity with predictable effect on further tumorigenesis.

Gan et al. have shown that MC-LR is able to enhance the stability of the Nrf2 transcription factor in the cytoplasm and its translocation to the nucleus via binding to the cytosolic regulator protein Keap1. Knockdown of Nrf2 mediated by siRNA can inhibit cell proliferation and cell cycle progression induced by MC-LR [78]. Therefore, upregulation of Nrf2 induced by MC-LR in tumor cells favors liver cancer cell growth. This study gives additional information supporting Nrf2 role in cancer tumorigenesis [78], respectively, of MC-LR. Moreover, a higher level of Nrf2 in toxin-treated rat primary hepatocytes after 48 h has been observed [79]. It is assumed that inhibition of protein phosphatases by MCs may affect the activity of DNA-dependent protein kinase (DNA-PK), an enzyme with key role in the nonhomologous terminal binding of DNA loops in the G_0-phase of the cell cycle observed in human lymphocytes [80].

Another important mechanism of genotoxicity is the impairment of DNA repair. Experimental animals exposed to sublethal low doses of MC have shown to develop tumorigenesis in coordination with the presence of dysfunctional p53 [81]. The increased formation of reactive oxygen species leads to oxidative DNA damage. A study shows that after 4 h of exposure to 0.01, 0.1, and 1 mg/mL of MC-LR DNA strand breaks were induced in dose dependent manner in human liver carcinoma cell line (HepG2 cells) [82]. Oxidized pyrimidines are repaired within a short time of exposure to MCs (8 h), while oxidized purines (mainly 8-hydroxyguanine) remain unrepaired in the DNA and accumulate [83] leading to GC-TA transversion mutations [84]. This statement has been verified *in vivo* with demonstrated elevation of 8-hydroxyguanine in male rat hepatocytes 24 h after treatment with 50 mg/

kg body weight of MC-LR [85]. In primary cultured rat liver cells exposed to NOD, the highest level of 8-oxo-2′-deoxyguanosine adducts is observed after 3 h exposure and its level decreased to control cells' levels after 24 h of exposure [85].

Many studies on MC-LR adverse effects establish its ability to change gene expression, and by these means contribute to a better understanding of MC-LR mechanisms linked to toxicity, genotoxicity, and carcinogenicity potential. Sueoka et al. [86] give the first evidence that MC-LR modulates the expression of tumor suppressor genes and oncogenes. They demonstrate that primary rat liver cells exposed to MC-LR (1 mM) for 6 h remarkably elevated the tumor necrosis factor α (TNF-α) expression, which could play the role of an endogenous tumor promoter [87]. The same study shows upregulation of early-response genes from the *jun* and *fos* gene families, proto-oncogenes, which are involved in gene regulation in response to different stimuli such as growth factors, cytokines, and viral and bacterial infections (**Table 1**).

In summary, one pathway of MC genotoxic activity is mediated by induction of ROS generation, thus leading to DNA strand breaks and at the same time significantly decreasing DNA repair system activity. The impairment of DNA repair together with DNA damage is an important factor involved in tumorigenesis. Chronic exposure to low concentrations of these cyanotoxins may increase the risk for carcinogenesis due to their potential long-term adverse effects (carcinogenic and genotoxic). For this reason, the International Agency for Research on Cancer classifies MC-LR as a possible human carcinogen [88]. **Figure 1** summarizes possible mechanisms of MR-LR genotoxicity with contribution to carcinogenesis.

Experimental model	Time/dose of exposure/method of administration	Main findings	Reference
Male Wistar rats	Single intravenous administration of MC-LR extract; 80 μg/kg body weight	The maximum MC-LR content (2.9% of the injected dose) detected 2 h after injection. Highest concentration found in kidney (0.034–0.295 μg/g dry weight); concentration in liver (0.003–0.052 μg/g dry weight).	[40]
Fish species *O. bonariensis* collected in Los Padres Lake (Argentina)	Fish residing in intoxicated water (MC-LR, -RR, -YR, and -LA total content in water: 2.8 ± 5.6 μg L^{-1})	Total content of MCs in liver (33.6 ± 37.2 μg kg^{-1}) is 10-fold higher than that in the fish muscles (3.9 ± 2.2 μg kg^{-1}).	[45]
Pigs (breed, PIC 337)	Oral administration of MC-LR in two treatment groups: 1. 0.04 μg MC-LR/kg body weight for 13 weeks 2. 2 μg toxin/kg body weight for 5 weeks	MC-LR not detected in serum; free MC-LR found in the large intestine (1.4 μg/kg dry weight) and kidney (1.9 μg/kg dry weight). The higher dosed animals accumulated MC-LR-conjugate in liver (26.4 μg/kg dry weight).	[52]
129-Trp53^{tm1Brd}N4 mice (homozygous p53 knockout B6 mice)	Intraperitoneal injection of MC-LR, 40 μg/kg body weight for 4, 24 h, and 4, 14, and 28 days	Increased proliferative response in liver after 28 days exposure time as detected by increased nuclear Ki-67 immunoreactivity and phosphohistone H3 expression.	[81]

Table 1.
Microcystins in animal studies.

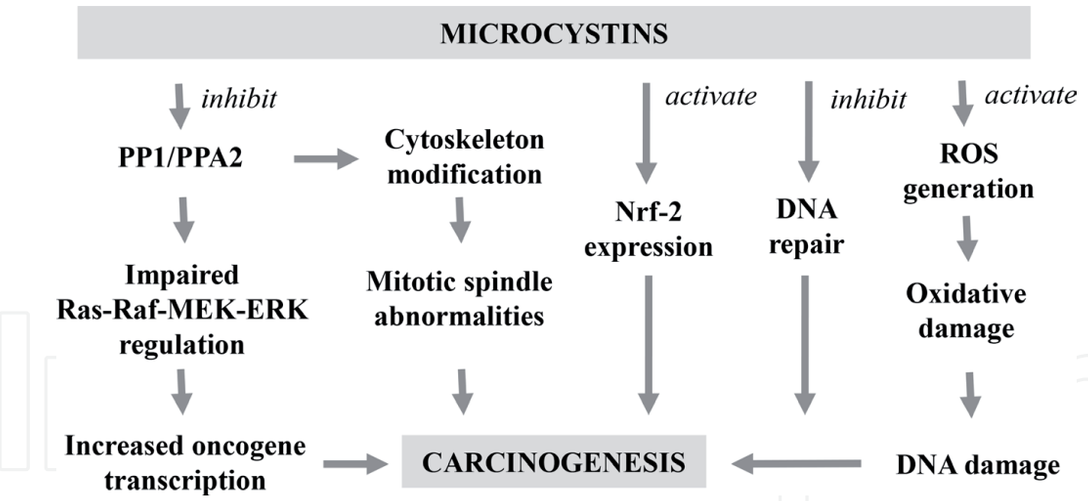

Figure 1.
Mechanisms of microcystin genotoxicity.

3.2 Cyclic peptides—potential sources of anticancer drugs

Essential for candidate molecules to be developed into useful anticancer therapeutics is their cancer selectivity. It is known that specific types of cancer can be targeted by redox-based therapies when cancer cells are assailable by increased ROS production induced by exogenous agents [89]. Thus, microcystin analogues are assumed to be selective anticancer drugs for certain types of cancer cells, specifically for those that express OATP, without causing significant toxicity to normal cells because of the differences of redox status between normal and cancer cells [90]. The development of OATP-targeting compounds based on the chemical structure of MC-LR, with unique physicochemical properties such as high water solubility, resistance to chemical hydrolysis or oxidation at near-neutral pH, and stability in pH shifts, appears to be a feasible and promising option in this direction [91]. There are studies focused on developing analogues of microcystin cyanotoxins for efficiently targeting the OATP-expressing metastatic cancers, which are resistant to conventional chemotherapy treatment [90] and many known cyanotoxins have been studied for their anticancer properties in human cell lines.

Currently, neither optimal nor targeted therapy has been developed for pancreatic cancer [92]. Overexpression of OATPs in pancreatic cancer offers an opportunity to develop effective novel cancer-targeted agents. A study demonstrates that MC-LR targeting OATP1B1 and OATP 1B3 can cause inhibition of proliferation of pancreatic cancer cells in a dose-response mode [92]. Study findings point that antiproliferative and pro-apoptotic effects are proportionally related to the expression of these transporters, thus suggesting an essential role for OATP expression in the process of MC-LR–induced cancer cell damage. Moreover, direct comparison of the inhibitory effect of MC-LR and the drug gemcitabine manifests a noticeable advantage of the toxin [92].

Monks et al. [93] have transfected cervical cancer cell line HeLa with the known OATP1B1 and OATP1B3 transporters seeking through appropriate *in vitro* models how MCs are uptaken into the cells and testing the activity of MCs against cells that express OATPs. Authors elucidate that transfected HeLa cells were 1000-fold more sensitive to MC-LR compared to the vector-transfected control cells, pointing that the expression of transporters imparts marked selectivity for MC cytotoxicity [93]. These observations suggest that MC cytotoxicity in OATP1B1- and OATP1B3-expressing HeLa cells is linked to cell-specific inhibition of PP2A and not to protein phosphatase inhibition in general.

These findings endorse the anticancer potential of MCs and raise hopes that cyanotoxins may have a promising future in cancer therapy. Challenges of potential organ-specific MC toxicity remain to be resolved by proper chemical modifications in the process of drug modulation.

4. Cylindrospermopsin—molecular mechanisms of toxicity and biotransformation

Humans are more susceptible to the exposure to cylindrospermopsin in comparison to other cyanotoxins because up to 90% of the total CYN is found outside the cyanobacterial cells [94]. Humpage et al. [95] recommend a maximum concentration of CYN in drinking water to be 1 μg/L based on tolerated daily intake, 0.03 μg/kg body weight.

CYN is generated by different freshwater cyanobacteria species, which are common worldwide, nowadays [96]. Many cyanotoxins are generally sequestered inside cyanobacteria until death, while cylindrospermopsin can be liberated in water during blooms [97].

CYN is a polycyclic uracil derivative containing guanidino and sulfate groups. The cyanobacterial toxin CYN is a tricyclic alkaloid that consists of a tricyclic guanidine moiety combined with hydroxymethyluracil. CYN has been recognized to induce cytotoxicity *in vitro* in human cell lines from liver and intestine [98]. The toxin primarily attacks the liver, but it is also a general cyanobacterial toxin that targets the spleen, kidney, heart, lungs, thymus, eyes, etc. [99].

The mechanisms of CYN toxicity and genotoxicity are not fully clarified. It is assumed that there are two types of toxic responses. It is established that CYN is more toxic in short-term (1–2 weeks) compared to long-term exposure in cell culture experiments [100]. Rapid toxicity is due to CYP450-generated metabolites [95]. The longer-term toxicity of CYN includes an irreversible inhibition of eukaryotic protein synthesis in *in vitro* experiments [98].

Many studies explore the cytotoxic effect of cylindrospermopsin and they are summarized in **Table 2**. These studies vary in type of cell culture used, time of exposure, concentrations of the CYN, and even the type of cytotoxicity test used.

Most *in vitro* experiments demonstrate that the cytotoxic effect of cylindrospermopsin is observed after long-term exposure (24–72 h). Toxicity of CYN on primary and carcinoma cell line is compared: the primary rat hepatocyte cells are more sensitive to the toxic effect of CYN, compared to KB cell line [101].

Morphological studies are more informative than cytotoxicity studies as they identify the types of cell damage. By means of microscopy, in that respect, pleomorphic nuclei, nucleolar segregation with altered nuclei, depraved Golgi apparatus, and apoptosis in human endothelial cells (HUVEC) after exposure to 0.375 μg/mL, CYN is observed [15]. The same authors also report morphological changes (mitochondrial damage, lipid degeneration, and nucleated segmentation with altered nuclei) in Caco-2 cells after exposure to a higher concentration of CYN (2.5 μg/mL). Absorption of CYN in Caco-2 cells is very limited, which explains the result [105]. Authors report that after cells being exposed to concentration of CYN 1–10 μM for 3, 10, and 24 h, the passage of CYN across the intestinal monolayer is about 2.5% after 3 h and increases slightly up to 20.5% after 24 h.

In search for possible mechanisms of CYN toxicity, it is observed that CYN significantly reduces GSH levels in rats' primary hepatocytes; a decline in the synthesis of GSH is the predominant mechanism, rather than an increased glutathione consumption [106], which could lead to increased oxidative stress. Other authors

Experimental model	Method applied	Experimental conditions	Results	Reference
Primary rat hepatocytes	MTT assay	0–10,000 ng/mL for 24, 48, 72 h exposure	The LC50 is 40 ng/mL; toxic effects are observed after 72 h.	[101]
Primary mouse hepatocytes	MTT assay	1–5 μM/mL	1–5 μM CYN induces concentration-dependent cytotoxicity in 18 h.	[102]
Primary human granulosa cells	MTT assay	0–1 μg/mL for 2, 4, 6, 24, 48, 72 h exposure	There is no effect when cells are exposed up to 1 μg/mL for short time (2–6 h). Cell viability is decreasing in a concentration-dependent way for longer time (24–72 h).	[103]
Hepatic cell lines: C3A and HepG2; colonic cell line: Caco-2	MTT assay	0.4–66 μM for 1, 2, 4, 6, 24 h exposure	The IC50 is 1.5 μM for C3A and HepG2 for 24 h exposure. The IC50 is 6.5 μM for Caco-2 for 25 h exposure.	[98]
CHO K1cells	Annexin V-FITC assay	0.1–10 μM for 12, 18, 24, 48 h exposure	Apoptosis is observed at low concentrations (1–2 μM) and short exposure (12 h). Necrosis is observed at higher concentrations (5–10 μM) and following longer exposure (24 or 48 h).	[60]
KB (human cervix carcinoma) cells	MTT assay	0–10,000 ng/mL for 24, 48, 72 h exposure	The LC50 is 200 ng/mL; toxic effects are observed after 72 h	[101]
HeLa cells	MTT assay	40, 20, 10, 5, 1, and 0.1 mg DW (lyophilized cyanobacterial biomass)/mL (cultivation medium) for 24 h of exposure	The IC50 is 0.2 ± 0.06 mg of lyophilized biomass per milliliter of culture medium.	[104]

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); KB (human cervix carcinoma) cells; C3A (human hepatocellular carcinoma); HepG2 (human liver hepatocellular carcinoma cell line); Caco-2 (human colorectal adenocarcinoma cell line); CHO-K1 (Chinese hamster ovary K1 cells); Annexin V-FITC (fluorescein isothiocyanate labeled annexin V); HeLa (human cervical epithelial adenocarcinoma).

Table 2.
In vitro cytotoxicity studies performed with cylindrospermopsin.

observe different and contradictory effects of CYN on the activity of gamma-glutamylcysteine synthetase (GCS)—the regulatory enzyme in GSH synthesis. In any case, reduction of GSH levels does not contribute significantly for the acute CYN toxicity *in vivo as presumed*, because no changes in the oxidative stress markers after exposure to CYN are evidenced [95].

The role of biotransformation of CYN is an important factor for understanding its toxic effect in cell lines, respectively, in various tissues. Scientific data show that toxicity and genotoxicity of CYN depend on cytochrome P450 (CYP)-mediated metabolism, as various CYP inhibitors can protect cells against toxicity, but it is not yet clear which isoforms are involved [95]. The higher activity of CYP450 in hepatocytes

involved in bioactivation events is found to be important for CYN toxicity in liver cell cultures [107]. Many *in vitro* and *in vivo* studies on the toxic mechanisms of CYN prove that metabolites of the toxin produced by CYP450 are mainly responsible for its toxicity, including its genotoxicity. Thus, the activity of the cytochrome P450 enzyme system has been investigated [102], demonstrating that the inhibition of CYP450 activity by proadifen or ketoconazole in mouse hepatocytes reduces the toxicity of CYN but does not alter its effect on protein synthesis. These observations may explain lower CYN toxicity in cell lines such as human cervix carcinoma (KB cells) [101], HeLa cell types [100], and CHO-K1 cells [108] compared to primary rat hepatocytes. Another study reveals that CYP1A1 and CYP1A2 are upregulated in human peripheral blood lymphocytes after CYN exposure [109].

Due to the simultaneous presence of different cyanobacterial toxins in aquatic environment, Hercog et al. [110] investigated the genotoxic potential of MC-LR and CYN mixtures, applied on HepG2 cells. Cells are treated with different doses of CYN, a single dose of MC-LR, and several combinations. A mixture of MC-LR and CYN provokes genotoxic damages, but to a lesser extent in case of strand breaks, compared to CYN itself. Data manifest that MC-LR provoke DNA strand breaks after short-term exposure, while CYN induces DNA damage after prolonged exposure in metabolically active cells [95, 111]. These data point that CYN exhibits higher genotoxic effects compared to MC-LR in the mixture of CYN and MC-LR. The same authors disclose mRNA expression levels of certain genes after 4 and 24 h of exposition to CYN or to MC-LRs, or to a combination of both cyanotoxins. Changes in the expression levels of genes involved in the metabolism of xenobiotic, genes involved in immediate-early response/signaling, and genes involved in response to DNA damage, upon exposure to CYN/MC-LR mixture, are not different to those induced by CYN itself [110], indicating higher genotoxicity of CYN.

In summary, the main target of CYN toxic activity is the liver, and CYN metabolism plays an important part for understanding the mechanisms of its toxicity. Therefore, the activity of CYP450 enzyme system is considered a key mechanism for CYN toxicity development in hepatocyte cultures, including genotoxicity. The higher sensitivity of liver cells exposed to CYN is due to bioactivation-dependent events, research indicates [105]. One of the CYN-known mechanisms is the irreversible inhibition of protein synthesis after long-term exposure [98]. Another mechanism is via oxidative stress induced by inhibiting the regulatory enzyme in GSH synthesis [106]. CYN induces DNA damage after longer exposure in metabolically active cells [95].

4.1 Anticancer properties of cylindrospermopsin

Caco-2 cells and HepG2 are often used human cell lines for cyanotoxin effects research. A study showed that CYN is linked to a variable effect in HepG2 cell line. Cyanotoxin diminishes lipid peroxidation in cells that have not been previously induced by phenobarbital exposure for 12 h and elevates it in phenobarbital-induced cells exposed to the highest CYN concentration (10 μg/L). Lipid peroxidation increases in both cell types after 24 h exposure, only at 10 μg/L CYN [112], demonstrating that the toxicity of low concentrations of CYN (<10 μg/L) is limited in human hepatoma cells. HepG2 cells are more sensitive compared to intestinal cells, while Caco-2 cells are even less sensitive. The observation is associated with the limited CYN uptake in colon cells as described by several authors [105, 113].

Oral intake is the major route of human exposure to CYN, which makes intestine a target organ. Huguet et al. [114] examined the cellular and molecular mechanisms of cylindrospermopsin toxicity on differentiated cell line of human intestinal Caco-2 cells. This cellular monolayer provides *in vitro* model performing functional

and morphological characteristics similar to those of enterocytes. Results reveal that differentiated Caco-2 cells exposed for 24 h to a subtoxic cylindrospermopsin concentration overexpress the gene products linked to DNA damage repair, including nucleosomal histone modifications [114]. Bain et al. examined the potential role of p53 tumor suppressor protein in CYN-induced gene expression in human hepatocellular carcinoma cell line [111]. Authors report that after 6 h of exposure to CYN, concentration-dependent increases in mRNA levels are observed for the p53 target genes CDKN1A, MDM2, GADD45alpha (all involved in the response to DNA damage), and BAX (involved in the apoptosis), indicating an early activation of p53 by CYN. The respective levels of mRNA for these genes remain elevated after 24 h. Data suggest that CYN can induce stress responses resulting in activation of the p53 transcription factor [111] and subsequent upregulation of DNA repair processes and activation of apoptosis.

That being said, a 72 h exposure of HepG2 cell line to CYN provokes DNA double strand breaks, providing evidence that CYN can perform as a direct genotoxin [115].

Obviously, available scientific data indicate controversial roles of CYN and its toxicity. CYN can cause severe cell damages, and it has the potential to activate DNA repair processes, which, concerning concentration and time-of-exposure–dependent activities, makes it another promising potential anticancer drug source.

5. Clinical toxicology and pharmacological aspects

Scientific paper analysis reveals some mechanisms linked to exposure to cyanotoxins and their effect on human health. Many episodes of severe poisonings have been registered after acute exposure, associated with adverse effects. Epidemiological studies reveal correlation between cyanotoxins and their toxic effects on human health [20].

Symptoms of poisoning by drinking water are much like those of gastrointestinal disturbances caused by a number of pathogenic bacteria, thus hampering differentiation of poisoning with cyanotoxins. Data for chronic exposure to low cyanobacterial toxin levels are still not well investigated.

Concerning the chemical diversity of cyanotoxins, the pathobiochemical mechanisms for cyanotoxin-associated diseases are variable [116] and the mechanism of toxicity is different. Thus, there is no universal antidote for treatment of cases of cyanobacteria intoxications. One treatment strategy is to apply chemoprotectants, especially for treatment of microcystin intoxications. However, there is less research available on cylindrospermopsin-induced toxicity treatment [99]. Commonly discussed is the application of antioxidants with vitamin E having the strongest protective effect, as oxidative stress is one of the most common pathobiochemical mechanisms of cyanobacteria intoxications. In reference to available knowledge about cellular uptake of cyanotoxins, especially microcystins, transport inhibitors are considered for potential administration in cases of cyanobacteria-related intoxications and in combination with other therapies. The antibiotic rifampin is reported as an example of such a drug approved for clinical use [117].

Due to nondefinitive manifestation of cyanotoxin poisoning, symptomatic treatment is applied, including oxygen application, aiming at respiratory distress amelioration, activated charcoal gastric lavage, forced diuresis for toxin elimination by glomerular filtration, alkalization, and hepatoprotective medication administration [118].

6. Discussion

Cyanobacterial blooms have been registered worldwide for centuries, and a correlation to associated human and animal illness has been suspected. Subsequently, it has been documented that blue-green algae produce various bioactive compounds with the common name cyanotoxins. Taking into account the wide variety of cyanobacteria, their effect on all aquatic ecosystems, and the various manifestation of cyanotoxin poisoning, it is essential to elucidate their toxicity mechanisms, as well as adequate treatment.

Most common and well-examined cyanotoxins are the ones of the microcystin family. Epidemiological studies do not provide definitive confirmation of linkage of acute or chronic exposure to cyanotoxins and human cancer development. Some animal studies demonstrate cyanotoxins' carcinogenic potential. Intraperitoneal injection of MC-LR in sublethal dose causes neoplastic nodules in mouse liver [119]. MC-LR application causes liver cancer in dose-dependent manner in rat model where protein phosphatase type 1 and type 2A activities' inhibition has been established [120]. Inhibition of serine/threonine phosphatase activity is a possible link between toxic and suspected carcinogenic potential of microcystins. Supporting evidences for this hypothesis, for example, are the findings that PP2A phosphatase inhibition by microcystins leads to disruption of MAPK signal transduction pathway [55]. This can be a possible explanation for increased proto-oncogene transcription observed [65] and promoted cell proliferation and tumorigenesis [66] in experiments with this kind of toxins. Considering research limitations of microcystin effects on humans, further investigations and evidence collection are needed to provide more robust correlation between cyanotoxin poisoning and cancer development.

CYN cancer potential has been less studied and not fully explained. It is assumed rather indirect. CYN metabolism and biotransformation with the participation of CYPs generate reactive metabolites and exhaust cellular glutathione. Thus, its toxicity and/or carcinogenic potential may be attributed to generated (geno)toxic metabolites and compromised antioxidant cellular defense.

Cellular penetration of MCs is mediated by tissue-specific OATPs. Overexpression of these transporters in certain cancer cells [92] provides an opportunity for the development of effective novel cancer-targeted agents. In support of this hypothesis, transfection of HeLa cancer cells with OATPs has been established to increase their susceptibility to MC treatment as compared to vector-transfected control cells [93]. More studies in this field are necessary to provide valuable data about MCs' application as anticancer remedies.

7. Conclusions

Cyanobacteria are proved in various habitats, such as drinking water reservoirs and recreational waters, at the basis of food chains, and thus, with a substantial impact on ecosystems and human health. Centurial observations of a correlation between water blooms and health issues in animals and humans are extended in numerous epidemiological, *in vivo* and *in vitro*, studies. Various bioactive compounds under the common name cyanotoxins are established as the reason for blooming water toxicity. Some of the toxic molecular mechanisms for certain cyanotoxins are clarified. Their bioavailability, metabolism, and biotransformation are proved as well. A possible link between cyanotoxin exposure and cancer development has been suspected and there is experimental research data in support of this hypothesis. Yet, cyanobacteria produce natural compounds with promising potential for the discovery of novel anticancer drugs. Improved alertness about cyanotoxin poisoning, its relation

to water blooms, poisoning symptoms, and specific treatment is needed in view of adequate human and animal health promotion and health care. Cyanobacteria have already been in the focus of food and pharmaceutical industry and cosmetics for a long time, incorporated in different preparations and food supplements. This requires raised awareness of the population and responsible institutions about the hazards of cyanotoxin contamination regarding food, water, and health remedies.

Acknowledgements

This study was supported by the Bulgarian National Science Fund, Grant No DN13/9, 15.12.2017; project "Cyanoprokaryotes—a new potential risk factor for cancer in Bulgaria?".

Author details

Deyana Georgieva Vankova[1*], Milena Gincheva Pasheva[1], Yoana Dimitrova Kiselova-Kaneva[1], Dobri Lazarov Ivanov[2] and Diana Georgieva Ivanova[1]

1 Department of Biochemistry, Molecular Medicine and Nutrigenomics, Faculty of Pharmacy, Medical University of Varna, Varna, Bulgaria

2 Department of Biology, Faculty of Pharmacy, Medical University of Varna, Varna, Bulgaria

*Address all correspondence to: deyana.vankova@mu-varna.bg

References

[1] Awramik SM. The oldest records of photosynthesis. Photosynthesis Research. 1992;**33**:75-89

[2] Zehr JP, Waterbury JB, Turner PJ, Montoya JP, Omoregie E, Steward GF, et al. Unicellular cyanobacteria fix N2 in the subtropical North Pacific Ocean. Nature. 2001;**412**:635-638

[3] Tan LT. Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry Reviews. 2007;**68**:954-979

[4] Meriluoto J, Blaha L, Bojadzija G, Bormans M, Brient L, Codd G, et al. Toxic cyanobacteria and cyanotoxins in European waters—Recent progress achieved through the CYANOCOST action and challenges for further research. Advances in Oceanography and Limnology. 2017;**8**(1):161-178

[5] Carmichael WW. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". Human and Ecological Risk Assessment. 2001;**7**:1393-1407

[6] Pitois S, Jackson MH, Wood BJB. Sources of the eutrophication problems associated with toxic algae: An overview. Journal of Environmental Health. 2001;**64**:25-32

[7] Dietrich D, Hoeger S. Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): A reasonable or misguided approach? Toxicology and Applied Pharmacology. 2005;**203**:273-289

[8] Martins JC, Vasconcelos VM. Microcystin dynamics in aquatic organisms. Journal of Toxicology and Environmental Health. 2009;**12**:65-82

[9] Rodrigues MA, Reis MP, Mateus MC. Liquid chromatography/negative electrospray ionization ion trap MS2 mass spectrometry application for the determination of microcystins occurrence in southern Portugal water reservoirs. Toxicon. 2013;**74**:8-18

[10] Jia J, Chen Q, Lauridsen TL. A systematic investigation into the environmental fate of microcystins and the potential risk: Study in Lake Taihu. Toxins. 2017;**8**(6):170

[11] Liu Y, Chen W, Li D, Huang Z, Shen Y, Liu Y. Cyanobacteria/cyanotoxin contaminations and eutrophication status before Wuxi drinking water crisis in Lake Taihu. Chinese Journal of Environmental Science. 2011;**23**:575-581

[12] Peng L, Liu Y, Chen W, Liu L, Kent M, Song L. Health risks associated with consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: Significance for freshwater aquacultures. Ecotoxicology and Environmental Safety. 2010;**73**:1804-1811

[13] Stoyneva-Gärtner MP, Descy JP, Latli A, Uzunov BA, Pavlova VT, Bratanova Z, et al. Assessment of cyanoprokaryote blooms and of cyanotoxins in Bulgaria in a 15-years period (2000-2015). Advances in Oceanography and Limnology. 2017;**8**(1):1-22

[14] Kaebernick M, Neilan BA. Ecological and molecular investigations of cyanotoxin production. FEMS Microbiology Ecology. 2001;**35**:1-9

[15] Gutiérrez-Praena D, Jos A, Pichardo S, Moreno IM, Cameán AM. Presence and bioaccumulation of microcystins and cylindrospermopsin in food and the effectiveness of some cooking techniques at decreasing their concentrations: A review. Food and Chemical Toxicology. 2013;**53**:139-152

[16] Messineo V, Melchiorre S, Di Corcia A, Gallo P, Bruno M. Seasonal succession of *Cylindrospermopsis raciborskii* and

Aphanizomenon ovalisporum blooms with cylindrospermopsin occurrence in the volcanic Lake Albano, Central Italy. Environmental Toxicology. 2010;**25**:18-27

[17] Saker ML, Metcalf JS, Codd GA, Vasconcelos VM. Accumulation and depuration of the cyanobacterial toxin cylindrospermopsin in the freshwater mussel Anodonta cygnea. Toxicon. 2004;**43**:185-194

[18] Cordeiro-Araújo MK, Chia MA, Bittencourt-Oliveira MC. Potential human health risk assessment of cylindrospermopsin accumulation and depuration in lettuce and arugula. Harmful Algae. 2017;**68**:217-223

[19] El-Deeb NM. Cyanobacterial toxin cylindrospermopsin: It's possible pathway from poisoning to cancer curing. Austin Biomolecules: Open Access. 2016;**1**(2):1009

[20] Svircev Z, Drobac D, Tokodi N, Mijovic B, Codd G, Meriluoto J. Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. Archives of Toxicology. 2017;**91**:621-650

[21] Wood R. Acute animal and human poisonings from cyanotoxin exposure—A review of the literature. Environment International. 2016;**91**:276-282

[22] Backer LC, Landsberg JH, Miller M, Keel K, Canine TKT. Cyanotoxin poisonings in the United States (1920s–2012)-review of suspected and confirmed cases from three data sources. Toxins. 2013;**5**(9):1597-1628

[23] Mulvenna V, Dale K, Priestly B, Mueller U, Humpage A, Shaw G, et al. Health risk assessment for cyanobacterial toxins in seafood. International Journal of Environmental Research and Public Health. 2012;**9**(3):807-820

[24] Roy-Lachapelle A, Solliec M, Bouchard MF, Sauvé S. Detection of cyanotoxins in algae dietary supplements. Toxins. 2017;**9**(3):76-93

[25] Chorus I, Bartram J. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and Management. In: Chorus I, Bertram J, editors. World Health Organization. Suffolk, Great Britain: St Edmundsbury Press; 1999. p. 416. Available from: http://www.who.int/iris/handle/10665/42827

[26] Francis G. Poisonous Australian lake. Nature. 1878;**18**:11-12

[27] Billings W. Public health and toxic cyanobacteria. Water-associated human illness in Northeast Pennsylvania and it suspected association with blue-green algae blooms. In: Carmichael WW, editor. Handbook of The Water Environment: Algal Toxins and Health. 1st ed. NY: Plenum Press; 1981. pp. 243-255

[28] Ueno Y, Nagata S, Tsutsumi T, Hasegawa A, Watanabe M, Park H, et al. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis. 1996;**17**(6):1317-1321

[29] Jochimsen EM, Carmichael WW, An JS, Cardo DM, Cookson ST, Holmes CE, et al. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. The New England Journal of Medicine. 1998;**338**:873-878

[30] Azevedo SMFO, Carmichael WW, Jochimsen EM, Rinehart KL, Lau S, Shaw GR, et al. Human intoxication by microcystins during renal dialysis treatment in Caruaru, Brazil. Journal of Toxicology. 2002;**181-182**:441-446

[31] Teixera M, Costa M, Carvalho V, Pereira M, Hage E. Gastroenteritis epidemic in the area of the Itaparica dam,

Bahia, Brazil. Bulletin of the Pan American Health Organization. 1993;**27**:244-253

[32] Turner P, Gammie A, Hollinrake K, Codd G. Pneumonia associated with contact with cyanobacteria. British Medical Journal. 1990;**300**:1440-1441

[33] Molica R, Azevedo S. Ecofisiologia de cianobactérias produtoras de cianotoxinas. Oecologia Australis. 2009;**13**:229-246

[34] Giannuzzi L, Sedan D, Echenique R, Andrinolo D. An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande dam Argentina. Marine Drugs. 2011;**9**:2164-2175

[35] Žegura B, Štraser A, Filipič M. Genotoxicity and potential carcinogenicity of cyanobacterial toxins—A review. Mutation Research. 2011;**727**:16-41

[36] Jaiswal KP, Yadav AS, Gothalwal R. Microcystins: An emerging biomarker and toxicity prediction in human and animal. International Journal of Biotech Trends and Technology. 2016;**17**(1):1-8

[37] WHO. Guidelines for drinking-water quality. In: Recommendations Chemical Fact Sheets. Vol. 1. Geneva, Switzerland: World Health Organization; 2004. pp. 407-408

[38] Obaidat A, Roth M, Hagenbuch B. The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. Annual Review of Pharmacology and Toxicology. 2012;**52**:135-151

[39] Cazenave J, Wunderlin DA, Bistoni MA, Ame MV, Krause E, Pflugmacher S, et al. Uptake, tissue distribution and accumulation of microcystin-RR in *Corydoras paleatus*, *Jenynsia multidentata* and *Odontesthes bonariensis*. Aquatic Toxicology. 2005;**75**:178-190

[40] Wang Q, Xie P, Chen J, Liang G. Distribution of microcystins in various organs (heart, liver, intestine, gonad, brain, kidney and lung) of Wistar rat via intravenous injection. Toxicon. 2008;**52**:721-727

[41] Fischer WJ, Altheimer S, Cattori V, Meier PJ, Dietrich DR, Hagenbuch B. Organic anion transporting polypeptides expressed in liver and brain mediate uptake of microcystin. Toxicology and Applied Pharmacology. 2005;**203**:257-263

[42] Ibelings BW, Chorus I. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: A review. Environmental Pollution. 2007;**150**:177-192

[43] Abe T, Unno M, Onogawa T, Tokui T, Kondo TN, Nakagomi R, et al. LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. Journal of Gastroenterology. 2001;**120**:1689-1699

[44] Cui Y, Konig J, Nies AT, Pfannschmidt M, Hergt M, Franke WW, et al. Detection of the human organic anion transporters SLC21A6 (OATP2) and SLC21A8 (OATP8) in liver and hepatocellular carcinoma. Laboratory Investigation. 2003;**83**(4):527-538

[45] Amé MV, Galanti LC, Menone ML, Gerpe MS, Moreno VJ, Wunderlin DA. Microcystin-LR, -RR, -YR and -LA in water samples and fishes from a shallow lake in Argentina. Harmful Algae. 2010;**9**:66-73

[46] Robinson NA, Pace J, Matson CF, Miura GA, Lawrence WB. Tissue distribution, excretion and hepatic biotransformation of microcystin-LR in mice. The Journal of Pharmacology and Experimental Therapeutics. 1990;**256**:176-182

[47] Pflugmacher S, Wiegand C, Oberemm A, Beattie KA, Krause E,

Codd GA, et al. Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: The first step of detoxication. Biochimica et Biophysica Acta. 1998;**1425**:527-533

[48] Kondo F, Matsumoto H, Yamada S, Ishikawa N, Ito E, Nagata S, et al. Detection and identification of metabolites of microcystins formed in vivo in mouse and rat livers. Chemical Research in Toxicology. 1996;**9**:1355-1359

[49] He J, Chen J, Xie P, Zhang D, Li G, Wu L, et al. Quantitatively evaluating detoxification of the hepatotoxic microcystins through the glutathione and cysteine pathway in the cyanobacteria-eating bighead carp. Aquatic Toxicology. 2012;**116**:61-68

[50] Li W, He J, Chen J, Xie P. Excretion pattern and dynamics of glutathione detoxification of microcystins in Sprague Dawley rat. Chemosphere. 2018;**191**:357-364

[51] Gehringer MM. Microcystin-LR and okadaic acid-induced cellular effects: A dualistic response. FEBS Letters. 2004;**557**:1-8

[52] Greer B, Meneel JP, Elliott CT. Uptake and accumulation of microcystin-LR based on exposure through drinking water: An animal model assessing the human health risk. Scientific Reports. 2018;**8**:491

[53] Swindle MM, Makin A, Herron AJ, Clubb FJJ, Frazier KS. Swine as models in biomedical research and toxicology testing. Veterinary Pathology. 2012;**49**:344-356

[54] Li W, Xie P, Chen J, He J, Guo X, Yu D, et al. Quantitative liquid chromatography-tandem mass spectrometry method for determination of microcystin-RR and its glutathione and cysteine conjugates in fish plasma and bile. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences. 2014;**963**:113-118

[55] Menezes C, Elisabete Valério E, Dias E. The Kidney Vero-E6 Cell Line: A Suitable Model to Study the Toxicity of Microcystins. Vol. 2. Rijeka, Croatia: InTechOpen; 2013. pp. 29-48

[56] Chen J, Xie P, Li L, Xu J. First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. Toxicological Sciences. 2009;**108**:81-89

[57] Ohta T, Sueoka E, Iida N, Komori A, Suganuma M, Nishiwaki R, et al. Nodularin, a potent inhibitor of protein phosphatases 1 and 2A, is a new environmental carcinogen in male F344 rat liver. Cancer Research. 1994;**54**:6402-6406

[58] Fastner J, Heinze R, Chorus I. Microcystin-content, hepatotoxicity and cytotoxicity of cyanobacteria in some German water bodies. Water Science and Technology. 1995;**32**:165-170

[59] Fischer A, Hoeger SJ, Stemmer K, Feurstein DJ, Knobeloch D, Nussler A, et al. The role of organic anion transporting polypeptides (OATPs/SLCOs) in the toxicity of different microcystin congeners in vitro: A comparison of primary human hepatocytes and OATP-transfected HEK293 cells. Toxicology and Applied Pharmacology. 2010;**245**:9-20

[60] Gacsi M, Antal O, Vasas G, Mathe C, Borbely G, Saker ML, et al. Comparative study of cyanotoxins affecting cytoskeletal and chromatin structures in CHOK1 cells. Toxicology In Vitro. 2009;**23**:710-718

[61] Piyathilaka MAPC, Pathmalal MM, Tennekoon KH, De Silva BGDNK, Samarakoon SR, Chanthirika S.

Microcystin-LR-induced cytotoxicity and apoptosis in human embryonic kidney and human kidney adenocarcinoma cell lines. Microbiologica. 2015;**161**:819-828

[62] Eriksson JE, Toivola D, Meriluoto JAO, Karaki H, Han YG, Hartshorne D. Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochemical and Biophysical Research Communications. 1990;**173**:1347-1353

[63] Buratti FM, Manganelli M, Vichi S, Stefanelli M, Scardala S, Testai M, et al. Cyanotoxins: Producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Archives of Toxicology. 2017;**91**:1049-1130

[64] Bagu JR, Sykes BD, Craig MM, Holmes CFB. A molecular basis for different interactions of marine toxins with protein phosphatase-1. The Journal of Biological Chemistry. 1997;**272**:5087-5097

[65] Li H, Xie P, Li G, Hao L, Xiong Q. In vivo study on the effects of microcystin extracts on the expression profiles of proto-oncogenes (c-fos, c-Jun and c-myc) in liver, kidney and testis of male Wistar rats injected i.v. with toxins. Toxicon. 2009;**53**:169-175

[66] Junttila MR, Li SP, Westermarck J. Phosphatase-mediated crosstalk between MAPK signalling pathways in the regulation of cell survival. The FASEB Journal. 2008;**22**:954-965

[67] Bouaïcha N, Maatouk I. Microcystin-LR and nodularin induce intracellular glutathione alteration, reactive oxygen species production and lipid peroxidation in primary cultured rat hepatocytes. Toxicology Letters. 2004;**148**:53-63

[68] Ding WX, Shen HM, Zhu HG, Ong CN. Studies on oxidative damage induced by cyanobacteria extract in primary cultured rat hepatocytes. Environmental Research. 1998;**78**:12-18

[69] Lankoff A, Banasik A, Nowak M. Protective effect of melatonin against nodularin-induced oxidative stress in mouse liver. Archives of Toxicology. 2002;**76**:158-165

[70] Pavagadhi S, Gong Z, Hande MP, Dionysiou DD, de la Cruz AA, Balasubramanian R. Biochemical response of diverse organs in adult Danio rerio (zebrafish) exposed to sub-lethal concentrations of microcystin-LR and microcystin-RR a balneation study. Aquatic Toxicology. 2012;**109**:1-10

[71] Valério E, Vasconcelos V, Campos A. New insights on the mode of action of microcystins in animal cells—A review. Medicinal Chemistry. 2016;**16**:1032-1041

[72] Lundqvist J, Pekar H, Oskarsson A. Microcystins activate nuclear factor erythroid 2-related factor 2 (Nrf2) in human liver cells in vitro—Implications for an oxidative stress induction by microcystins. Toxicon. 2017;**126**:47-50

[73] Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. The Journal of Biological Chemistry. 1999;**274**:33627-33636

[74] Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes and Development. 1999;**13**:76-86

[75] Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Molecular Cell. 2006;**21**:689-700

[76] Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, et al. Dysfunctional Keap1-Nrf2 interaction in non-small-cell lung cancer. PLoS Medicine. 2006;**3**(10):1865-1876

[77] Homma S, Ishii Y, Morishima Y, Yamadori T, Matsuno Y, Haraguchi N, et al. Nrf2 enhances cell proliferation and resistance to anticancer drugs in human lung cancer. Clinical Cancer Research. 2009;**15**:3423-3432

[78] Nanqin G, Xiaoyun S, Lirong S. Activation of Nrf2 by microcystin-LR provides advantages for liver cancer cell growth. Chemical Research in Toxicology. 2010;**23**:1477-1484

[79] López-Alonso H, Rubiolo JA, Vega F, Vieytes MR, Botana LM. Protein synthesis inhibition and oxidative stress induced by cylindrospermopsin elicit apoptosis in primary rat hepatocytes. Chemical Research in Toxicology. 2013;**26**:203-212

[80] Lankoff A, Bialczyk J, Dziga D, Carmichael WW, Gradzka I, Lisowska H, et al. The repair of gamma-radiation-induced DNA damage is inhibited by microcystin-LR, the PP1 and PP2A phosphatase inhibitor. Mutagenesis. 2006;**21**:83-90

[81] Clark SP, Ryan TP, Searfoss GH, Davis MA, Hooser SB. Chronic microcystin exposure induces hepatocyte proliferation with increased expression of mitotic and cyclin-associated genes in P53-deficient mice. Toxicologic Pathology. 2008;**36**:190-203

[82] Zegura B, Sedmak B, Filipic M. Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2. Toxicon. 2003;**41**:41-48

[83] Zegura B, Lah TT, Filipic M. The role of reactive oxygen species in microcystin-LR-induced DNA damage. Toxicology. 2004;**200**:59-68

[84] Grollman AP, Moriya M. Mutagenesis by 8-oxoguanine: An enemy within. Trends in Genetics. 1993;**9**:246-249

[85] Maatouk I, Bouaïcha N, Plessis MJ, Périn F. Detection by 32P-postlabelling of 8-oxo-7 8-dihydro-20-deoxyguanosine in DNA as biomarker of microcystin-LR and nodularin-induced DNA damage in vitro in primary cultured rat hepatocytes and in vivo in rat liver. Mutation Research. 2004;**564**(1):9-20

[86] Sueoka E, Sueoka N, Okabe S, Kozu T, Komori A, Ohta T, et al. Expression of the tumor necrosis factor a gene and early response genes by nodularin, a liver tumor promoter, in primary cultured rat hepatocytes. Journal of Cancer Research and Clinical Oncology. 1997;**123**:413-419

[87] Komori A, Yatsunami J, Suganuma M, Okabe S, Abe S, Sakai A, et al. Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation. Cancer Research. 1993;**53**:1982-1985

[88] IARC. Cyanobacterial Peptide Toxins. 2006. Available from: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono94-7.pdf

[89] Wang J, Yi J. Cancer cell killing via ROS: To increase or decrease, that is the question. Cancer Biology & Therapy. 2008;7(12):1875-1884

[90] Sainis I, Fokas D, Vareli K, Tzakos AG, Kounnis V, Briasoulis E. Cyanobacterial cyclopeptides as lead compounds to novel targeted cancer drugs. Marine Drugs. 2010;**8**(3):629-657

[91] Zhang D, Xie P, Chen J. Effects of temperature on the stability of microcystins in muscle of fish and its consequences for food safety. Bulletin of Environmental Contamination and Toxicology. 2010;**84**:202-207

[92] Kounnis V, Chondrogiannis G, Mantzaris MD, Tzakos AG, Fokas D, Papanikolaou NA, et al. Microcystin LR shows cytotoxic activity against pancreatic cancer cells expressing the membrane OATP1B1 and OATP1B3 transporters. Anticancer Research. 2015;**35**(11):5857-5865

[93] Monks NR, Liu S, Xu Y, Yu H, Bendelow AS, Moscow JA. Potent cytotoxicity of the phosphatase inhibitor microcystin LR and microcystin analogues in OATP1B1- and OATP1B3-expressing HeLa cells. Molecular Cancer Therapeutics. 2007;**6**:587-598

[94] Rücker J, Stüken A, Nixdorf B, Fastner J, Chorus I, Wiedner C. Concentrations of particulate and dissolved cylindrospermopsin in 21 aphanizomenon-dominated temperate lakes. Toxicon. 2007;**50**:800-809

[95] Humpage AR, Fontaine F, Froscio S, Burcham P, Falconer IR. Cylindrospermopsin genotoxicity and cytotoxicity: Role of cytochrome P-450 and oxidative stress. Journal of Toxicology and Environmental Health Part A: Current Issues. 2005;**68**:739-753

[96] Kinnear S. Cylindrospermopsin: A decade of progress on bioaccumulation research. Marine Drugs. 2010;**8**:542-564

[97] Chiswell RK, Shaw GR, Eaglesham G, Smith MJ, Norris RL, Seawright AA, et al. Stability of cylindrospermopsin, the toxin from the cyanobacterium, cylindrospermopsis raciborskii: Effect of pH, temperature, and sunlight on decomposition. Environmental Toxicology. 1999;**14**:155-161

[98] Froscio SM, Fanok S, Humpage AR. Cytotoxicity screening for the cyanobacterial toxin cylindrospermopsin. Journal of Toxicology and Environmental Health Part A: Current Issues. 2009;**72**:345-349

[99] Guzmán-Guillén R, Prieto AI, Moyano R, Blanco A, Vasconcelos V, Cameán AM. Dietary l-carnitine prevents histopathological changes in tilapia (*Oreochromis niloticus*) exposed to cylindrospermopsin. Environmental Toxicology. 2017;**32**:241-254

[100] Shaw GR, Seawright AA, Moore MA, Lam PKS. Cylindrospermopsin, a cyanobacterial alkaloid: Evaluation of its toxicological activity. Therapeutic Drug Monitoring. 2000;**22**:89-92

[101] Chong MWK, Wong BSF, Lam PKS, Shaw GR, Seawright AA. Toxicity and uptake mechanism of cylindrospermopsin and lophyrotomin in primary hepatocytes. Toxicon. 2002;**40**:205-211

[102] Froscio SM, Humpage AR, Burcham PC, Falconer IR. Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. Environmental Toxicology. 2003;**18**:243-251

[103] Young FM, Micklem J, Humpage AR. Effects of blue-green algal toxin cylindrospermopsin (CYN) on human granulosa cells in vitro. Reproductive Toxicology. 2008;**25**:374-380

[104] Hrouzek P, Kapuścik A, Vacek J, Voráčová K, Paichlová J, Kosina P, et al. Cytotoxicity evaluation of large cyanobacterial strain set using selected human and murine in vitro cell models. Ecotoxicology and Environmental Safety. 2016;**124**:177-185

[105] Fernández DA, Louzao MC, Vilariño A, Fraga M, Espiña B, Vieytes MR, et al. Evaluation of the intestinal permeability and cytotoxic effects of cylindrospermopsin. Toxicon. 2014;**91**:23-34

[106] Runnegar MT, Kong S, Berndt N. Protein phosphatase inhibition and in vivo hepatotoxicity of microcystins.

American Journal of Physiology. Gastrointestinal and Liver Physiology. 1993;**265**:224-230

[107] Bazin E, Mourot A, Humpage AR, Fessard V. Genototoxicity of a freshwater cyanotoxin cylindrospermopsin, in two human cell lines: Caco-2 and HepaRG. Environmental and Molecular Mutagenesis. 2010;**51**:251-259

[108] Lankoff A, Wojcik A, Lisowska H, Bialczyk J, Dziga D, Carmichael WW. No induction of structural chromosomal aberrations in cylindrospermopsin-treated CHO-K1 cells without and with metabolic activation. Toxicon. 2007;**50**:1105-1115

[109] Straser A, Filipic M, Zegura B. Cylindrospermopsin induced transcriptional responses in human hepatoma HepG2 cells. Toxicology In Vitro : An International Journal Published in Association with BIBRA. 2013;**27**:1809-1819

[110] Hercog K, Maisanaba S, Filipic M, Jos A, Cameánn AM, Zegura B. Genotoxic potential of the binary mixture of cyanotoxins microcystin—LR and cylindrospermopsin. Chemosphere. 2017;**189**:319-329

[111] Bain P, Shae G, Patel B. Induction of P53-regulated gene expression in human cell lines exposed to the cyanobacterial toxin cylindrospermopsin. Journal of Toxicology and Environmental Health Part A: Current Issues. 2007;**70**:1687-1693

[112] Liebel S, Oliveira Ribeiro CA, Magalhaes VF, Silva C, Ramsdorf WA, Rossi SC, et al. Low concentrations of cylindrospermopsin induce increases of reactive oxygen species levels, metabolism and proliferation in human hepatoma cells (HepG2). Toxicology In Vitro. 2015;**29**:479-488

[113] Pichardo S, Cameán AM, Jos A. In vitro toxicological assessment of cylindrospermopsin: A review. Toxins. 2017;**9**(402):1-29

[114] Huguet A, Hatton A, Villot R, Quenault H, Blanchard Y, Fessard V. Modulation of chromatin remodelling induced by the freshwater cyanotoxin cylindrospermopsin in human intestinal Caco-2 cells. PLoS One. 2014;**9**(6):99-121

[115] Alja Š, Filipič M, Novak M, Žegura B. Double strand breaks and cell-cycle arrest induced by the cyanobacterial toxin cylindrospermopsin in HepG2 cells. Marine Drugs. 2013;**11**(8):3077-3090

[116] Facciponte DN, Bough MW, Seidler D, Carroll JL, Ashare A, Andrew AS, et al. Identifying aerosolized cyanobacteria in the human respiratory tract: A proposed mechanism for cyanotoxin-associated diseases. Science of the Total Environment. 2018;**645**:1003-1013

[117] Dawson RM. The toxicology of microcystins. Toxicon. 1998;**36**(7):953-962

[118] Zlateva S. Poisoning with molluscs and crustaceans. In: Kanev K, Stavrev D, editors. Handbook of Marine Toxicology. Poisoning with Sea Food and Marine Animals Incidence. 1st ed. Medical University Varna: Publishing Steno; 2018. p. 26. ISBN 978-619-221-163-0. IBSN 978-954-449-977-8

[119] Ito E, Kondo F, Terao K, Harada KI. Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin-LR. Toxicon. 1997;**35**(9):1453-1457

[120] Nishiwaki-Matsushima R, Oht T, Nishiwaki S, et al. Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. Journal of Cancer Research and Clinical Oncology. 1992;**118**(6):420-424