Toxicology Reports 3 (2016) 180–189

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Cyanotoxins at low doses induce apoptosis and inflammatory effects in murine brain cells: Potential implications for neurodegenerative diseases

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ARTICLE INFO

Article history:
Received 1 November 2015
Received in revised form
17 December 2015
Accepted 24 December 2015
Available online 15 January 2016

Keywords:
Cyanotoxins
Low doses
Apoptosis
Inflammation
Brain cells
Neurodegenerative diseases

ABSTRACT

Cyanotoxins have been shown to be highly toxic for mammalian cells, including brain cells. However, little is known about their effect on inflammatory pathways. This study investigated whether mammalian brain and immune cells can be a target of certain cyanotoxins, at doses approximating those in the guideline levels for drinking water, either alone or in mixtures. We examined the effects on cellular viability, apoptosis and inflammation signalling of several toxins on murine macrophage-like RAW264.7, microglial BV-2 and neuroblastoma N2a cell lines. We tested cylindrospermopsin (CYN), microcystin-LR (MC-LR), and anatoxin-a (ATX-a), individually as well as their mixture. In addition, we studied the neurotoxins β-*N*-methylamino-L-alanine (BMAA) and its isomer 2,4-diaminobutyric acid (DAB), as well as the mixture of both. Cellular viability was determined by the MTT assay. Apoptosis induction was assessed by measuring the activation of caspases 3/7. Cell death and inflammation are the hallmarks of neurodegenerative diseases. Thus, our final step was to quantify the expression of a major proinflammatory cytokine TNF-α by ELISA. Our results show that CYN, MC-LR and ATX-a, but not BMAA and DAB, at low doses, especially when present in a mixture at threefold less concentrations than individual compounds are 3–15 times more potent at inducing apoptosis and inflammation. Our results suggest that common cyanotoxins at low doses have a potential to induce inflammation and apoptosis in immune and brain cells. Further research of the neuroinflammatory effects of these compounds in vivo is needed to improve safety limit levels for cyanotoxins in drinking water and food.

1. Introduction

The quality of drinking water is a major worldwide public health issue. A large number of man-made chemicals and naturally occurring toxins have been detected in many drinking water sources. Freshwater cyanobacteria may accumulate in surface water supplies as "blooms" and may concentrate on the surface as blue–green "scums". They produce a range of toxic compounds called cyanotoxins that have a deleterious effect on raw water sources [53].

Cyanobacterial blooms are occurring with increasing frequency worldwide in fresh, brackish and marine water and in North America, Europe, Australia, Asia, Africa, Indian and Pacific oceans [10,14,1,49,9,35]. For instance, over the last decade, in Quebec (Canada), the number of affected water bodies increased from 34 in 2004 to 119 in 2009 [36,37] and is expected to continue to increase. Risk management of emerging cyanotoxins in worldwide water sources is compromised due to insufficient human and experimental data [11]. And, it is clear that current water quality guidelines, which focus on individual toxins, do not consider the potential combined or synergistic effects of mixtures of toxins.

Cyanotoxins are resistant to chemical and biological degradation and bioaccumulate in the food chain [32,25]. They can be classified into three main types according to their mechanism of action such as hepatotoxins (e.g., microcystins, cylindrospermopsin), neurotoxins (e.g., anatoxins), dermatotoxins or skin irritants. Both hepatotoxins and neurotoxins are produced by cyanobacteria commonly found in surface water and therefore are of relevance to drinking water supplies [57,10]. Thus, our interest in this study is to focus on these cyanotoxins (see Table 1). We believe that the classification of cyanotoxins by target organ (i.e., hepatotoxins or neurotoxins) is clearly not up to date, given the

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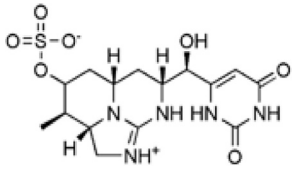
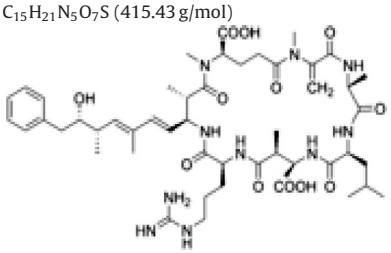
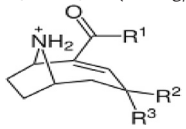
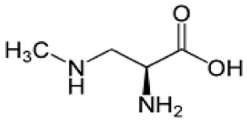
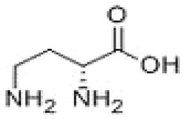
http://dx.doi.org/10.1016/j.toxrep.2015.12.008

Table 1
Cyanotoxins used in this study (abbreviations, molecular formulas, and brief descriptions), which are known to act as hepatotoxins or neurotoxins.

Group of cyanotoxin	Name (abbreviation)	Chemical and molecular formula	Description
Group A	Cylindrospermopsin (CYN)	 $C_{15}H_{21}N_5O_7S$ (415.43 g/mol)	CYN is a zwitterionic tricyclic alkaloid produced by a variety of freshwater cyanobacteria. It is a protein synthesis inhibitor, may be carcinogenic, and inhibits pyrimidine nucleotide synthesis. It is classified as a cytotoxin and hepatotoxin
	Microcystin-LR (MC-LR)	 $C_{49}H_{74}N_{10}O_{12}$ (995.2 g/mol)	MC-LR is a cyclic heptapeptide produced by cyanobacteria. It is considered to be the most toxic compound of the microcystin family. It is a tumor promoter and selective inhibitor of protein phosphatase 1 (PP1) and 2A (PP2A). It is classified as a hepatotoxin
	Anatoxin-a (ATX-a)	 $C_{10}H_{15}NO$ (281.3 g/mol)	ATX-a, also known as Very Fast Death Factor, is a secondary, bicyclic amine alkaloid and cyanotoxin with acute neurotoxicity. It was first discovered in the early 1960s in Canada, and was isolated in 1972. It is a nicotinic acetylcholine receptor ligand
Group B	β-*N*-methylamino-L-alanine (BMAA)	 $C_4H_{10}N_2O_2$ (154.60 g/mol)	BMAA is a non-proteinogenic amino acid produced by cyanobacteria and cycads. It is considered a possible cause of ALS/PDC (amyotrophic lateral sclerosis/parkinsonism–dementia complex) and its toxicity has been associated with possible chronic neurodegeneration. It is classified as a neurotoxin
	2,4-Diaminobutyric acid (DAB)	 $C_4H_{10}N_2O_2$ (191.06 g/mol)	DAB is a structural isomer of BMAA and is classified as a neurotoxin.

available knowledge on their toxic mechanisms, which are relevant for several cell types.

The hepatotoxin, microcystin-LR (MC-LR), was the first microcystin chemically identified and the most studied to date. It has been associated with most of the incidents of toxicity involving microcystins in several countries [10,25]. MC-LR is inhibitor of protein phosphatases 1 and 2A, which are present in virtually all cells and involved in several cell processes including cell division and apoptosis, induces sustained phosphorylation of proteins in cells and DNA damage, and is a potent tumor promoter in chronic exposures [21,49]. The tricyclic guanidinic alkaloid cylindrospermopsin (CYN) is also classified as a hepatotoxin but has a different mechanism of action, being a protein and glutathione synthesis inhibitor, with a major impact on liver cells. Indeed, CYN or its metabolite(s) have been shown in vivo to form adducts in liver DNA in mice [47,20,22]. The binding of cylindrospermopsin or a metabolite to DNA, and possibly to RNA, in liver cells has been proposed as a possible mechanism for the inhibition of protein synthesis that occurs with cylindrospermopsin toxicity but also in other organs such as kidneys, spleen, intestine, thymus and heart in vertebrates [13,14]. In contrast to neurotoxic alkaloids, CYN acts more slowly, taking about 5–6 days to kill mice with a LD50 of 200 μg/kg [12]. It has caused serious gastrointestinal and respiratory distress through exposure via drinking water in humans and domestic animals [8,14].

In general, the members of the neurotoxin category are not considered to widespread as hepatotoxins in water supplies, and they are not considered to pose the same degree of risk from chronic exposure as microcystins [57]. Anatoxin-a (ATX-a) was the first cyanotoxin belonging to the class of the neurotoxins to be characterized [7]. This alkaloid is a potent post-synaptic cholinergic nicotinic agonist and neuromuscular blocking agent that is a highly toxic nerve poison but has a short biological half-life [12]. The Guideline value (GV) for drinking water was established at 12.24 μg/l [18].

β-*N*-methylamino-L-alanine (BMAA) has been detected in several resource waterbodies [38] and was the first neurotoxin isolated from cycad trees on Guam in 1967. It may be involved in multiple neurodegenerative diseases [16] such as amyotrophic lateral sclerosis/parkinsonism–dementia complex (ALS/PDC) on the Island of Guam in the East Pacific [3]. BMAA has also been detected in the brains of Canadian patients with Alzheimer's disease [41]; however, others have failed to detect BMAA in brains of patients with neurodegenerative diseases [39]. BMAA has an structural isomer, the 2,4-diaminobutyric acid (DAB) which is very similar to the non-essential amino acid alanine.

It is well known that neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases are associated with inflammation processes [31]. Inflammation is a rapid response of tissue to injury and is characterized in the acute phase by increased