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RESEARCH

Comparative cytotoxicity of selected cyanotoxins on Human Embryonic Kidney (HEK-293) and Human Kidney Adenocarcinoma (ACHN) Cells.

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ABSTRACT

The aim of present study was to evaluate the cytotoxic effects of some selected cyanotoxins namely, MC-LR, MC-RR, MC-LF, MC-LW and Nodularin on normal human kidney cells (HEK-293) and human kidney adenocarcinoma cells (ACHN). Cells were exposed to different concentrations of (1.0–200 µM) cyanotoxin variants for 24 h and the cytotoxic effects were evaluated by Sulphorhodamine B (SRB) assay. Overall findings of the study demonstrate that cyanotoxins could cause cytotoxic effects on kidney cells. MC-LR was the most toxic while MC-LW was least toxic cyanotoxin on both cell types tested. MC-RR, MC-LF and Nodularin had moderate cytotoxicity on human renal cells.

INTRODUCTION

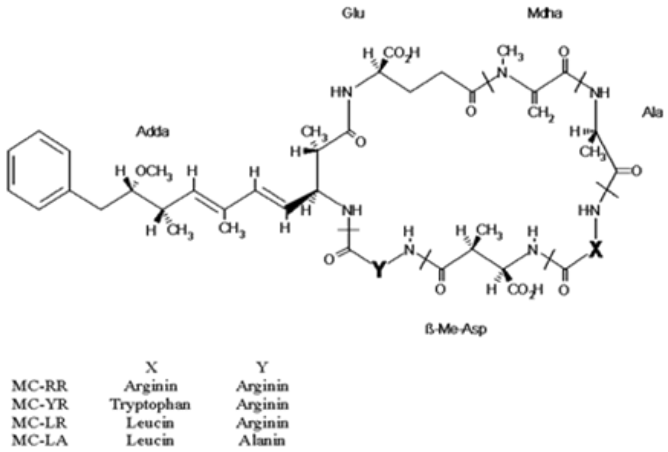
The cyanobacteria are frequently found in marine, brackish and freshwaters, including lakes, ponds, rivers, hot springs and reservoirs. Similar to the marine algal blooms, cyanobacteria will periodically grow exuberantly, known as “blooms” (Xu et al., 2011). These blooms can cause significant environmental impacts such as deterioration of water quality, depletion of underline water, subsequent fish kills, formation of foul odour and production to toxic substances (Bownic, 2010).

When cyanobacteria produce highly active biotoxins, the blue green algal blooms are known as a “harmful algal bloom (HAB)” (Carmichael, 2001). Cyanobacteria produce a supreme array of bioactive secondary metabolites, including alkaloids, polyketides and non-ribosomal peptides considered as cyanotoxins (Nogueira et al., 2004). It was documented that when cyanobacteria cell density exits more than 75% in the aquatic environment the secondary metabolites produce by the particular toxic cyanobacteria can cause toxic effects on organisms (Ernst et al., 2005). Health hazards to livestock, wildlife and human intoxications due to toxic cyanobacteria have been documented (Feurstein et al., 2009). Under circumstances of excessive cyanobacterial growth these toxins can be accumulated in aquatic organisms and transferred to higher tropic levels as well (Magalhaes et al., 2003).

Thus, the contamination of natural water bodies by cyanotoxins produced by cyanobacterial blooms is a worldwide problem challenging the supply of safe drinking water (Feurstein et al., 2009). The toxins of freshwater cyanobacteria are classified into two groups, neurotoxins and hepatotoxins, which include cyclic-peptide microcystins (MC) and nodularin (Frank, 2002; Magalhaes et al., 2003) which are the commonest and most abundant cyanotoxins in freshwater (Fischer and Dietrich, 2000; Gupta et al., 2003). Other cyanotoxins include anatoxin-a, anatoxin-as, aplysiatoxin, cylindrospermopsin, domoic acid, nodularin R and saxitoxin (Gremberghe et al., 2009; Hitzfeld et al., 2000; Schatz et al., 2007).

Generalized format of MC is Cyclo (D-Ala1-X2-D-MeAsp3-Y4-Adda-Arg5-D-Glu6-Mdha7-) where, X and Y are variable amino acids, D-MeAsp is D-erythro-bmethylaspartic acid, Adda is (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2-6-8-trimethyl 10-phenyldeca-4.6-dienoic acid and Mdha is N-methyldehydroalanine (Feurstein et al., 2011; Chen et al., 2004). The unusual amino acid Adda is essential for expression of biological activity. Combinations of the two variable L-amino acids, X and Y, account for many of the microcystin variants and are used in the nomenclature of the toxins. The XY variable

amino acids for MC-LR, MC-RR and MC-YR are leucine (L), arginine (R) and tyrosine (Y) (Gupta et al., 2003).



	X	Y
MC-RR	Arginin	Arginin
MC-YR	Tryptophan	Arginin
MC-LR	Leucin	Arginin
MC-LA	Leucin	Alanin

Figure 1: Chemical structure of MC molecule with variable amino acid molecules in X and Y positions (Source: Gupta et al., 2003)

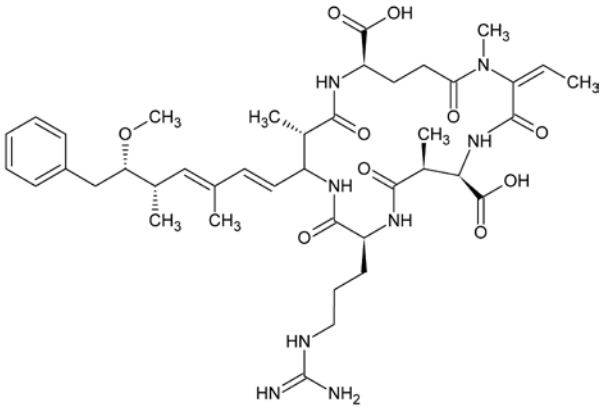


Figure 2: Chemical structure of Nodularin molecule (Source: Dittmann et al., 2013)

Public awareness regarding the cyanotoxins arose after number of intoxication scenarios reported in different parts of the world (Chorus, 2002). Severe hepatotoxicity of these toxins has been confirmed (Gupta et al., 2003). MCs are mainly excreted by the hepatocytes, but up to 9% can be eliminated by urine (Bischoff, 2001) which makes the kidneys a potential target for cyanotoxin toxicity (Menezes et al., 2013). Thus the kidney might also be an important target organ for MCs (Dias et al., 2009).

Present study was carried out to evaluate cytotoxic effects of some selected MCs (MC-LR, MC-RR, MC-LF and MC-LW) and Nodularin on human embryonic kidney cells (HEK-293) and human kidney adenocarcinoma cells (ACHN).

MATERIALS AND METHODS

Cell culture

Human cell cultures [ACHN (Human renal adenocarcinoma; Catalog number CRL-1611™) and HEK-293 (Human embryonic kidney; Catalog number CRL-1573™)] cell lines and reagents for cell culture experiments [Eagles Minimum Essential Medium (EMEM)] were purchased from American Type Culture Collection (ATCC), USA. TRIzol reagent (15596-018) was purchased from Invitrogen Life Technologies, USA while, Fetal Bovine Serum (FBS), Trypsin-EDTA, Strep-penicillin and all other chemicals were purchased from Sigma-Aldrich, USA.

Complete growth medium for both cell cultures was prepared by adding FBS and Strep-penicillin to make final concentration in the medium at 10% and 0.1% respectively. Maintenance of cell cultures was done at 37°C temperature with 5% CO_2 /air and 90 % ± 5 % humidity. Culture medium was changed every 2/3 days.

Cytotoxicity assay

Each type of cells was seeded at 5×10^3 cells per well into a 96well plate and incubated 24h for attachment. HEK-293 and ACHN cells were treated with different concentrations of MC-LR, MC-RR, MC-LF, MC-LW and Nodularin (1.0–200 µM) for 24 h and cytotoxicity was evaluated by Sulphorhodamine B (SRB) assay as described previously (Samarakoon et al., 2010).

SRB assay

After 24h incubation, cells were briefly washed with 1 x Phosphate Buffered Saline and fresh medium was placed in each well. Ice cold 50 % Tri Chloro Acetic Acid (25 µL) was layered on top of the fresh medium overlaying the cells and incubated at 4 °C for one hour to ensure cell fixation. The cells were then washed 5 times with tap water. The plate was air dried and the fixed cells were stained with 0.4 % (w/v) SRB dissolved in 1 % acetic acid for 15 min at room temperature. Then the plate was quickly washed five times with 1 % acetic acid and air dried. Finally, 200 µL of unbuffered Tris-base solution (pH 7.5) was added to the each well and the plate was placed on a plate shaker for 30 min at room temperature. Plate was then read at optical density (OD) 540 nm, using a microplate reader (ELx 800 Universal Microplate Reader, BIO-TEK instruments, USA). The results were expressed as a percentage of control values.

Each assay was performed in triplicate in three different experiments. The half maximal inhibitory concentration (IC_{50}) values for ACHN and HEK-293 cells for 24 h exposure to MC-LR were determined by analyzing percentage of control values in each cytotoxicity assay with sigmoid dose-response inhibition curves using GraphPad Prism software (version 5.0).

RESULTS

A significant cytotoxicity was induced in both types of HEK-293 and ACHN cells by the cyanotoxins MC-LR, MC-RR, MC-LF, MC-LW and Nodularin. When comparing the IC_{50} value of each type of toxin on each type of cell, all the toxins had a significantly higher cytotoxicity on normal kidney cells than on the kidney adenocarcinoma cells. Above statement was proven by the lower IC_{50} value for HEK-293 cell for each type of toxin. Furthermore, MC-LR had the lowest IC_{50} values for both types of cells (16.57±0.035 for HEK-293 and 62.36±0.037 for ACHN cells) while MC-LW had the highest IC_{50} values for both types of cells (1158.16±9.025 for HEK-293 and 1589.78±3.206 for ACHN cells). Table 1 summarizes the results.

Table 1: IC_{50} values of different types of cyanotoxins for HEK-293 and ACHN cell lines.

Cyanotoxin type	IC_{50} µM	
	HEK-293	ACHN
MC-LR	16.57±0.035	62.36±0.037
MC-RR	85.96±2.296	159.14±1.160
MC-LF	1158.16±9.025	1589.78±3.206
MC-LW	1068.09±6.148	1268.76±6.143
Nodularin	58.96±1.256	98.34±0.978

DISCUSSION

Cyanotoxin induced toxic effects on human liver is well documented (Zegura et al., 2003) but, an increasing number of recent publications emphasized the need of thorough evaluation of their effects on other vital organs. The adverse effects of cyanotoxins in distinct organs is an important issue for risk assessment, because the guideline values for some types of cyanotoxins in drinking water (Ex: for MC-LR, 1nM) are still provisional values, based on limited toxicological data (WHO, 2009). The exposure to low doses of cyanotoxins corresponds to the most practical kidney intoxication scenario, considering that it is not the main target organ of this toxin. However, the role of the kidney in toxin elimination might expose the kidney cells to a low internal dose that can be biologically effective in the induction of nephrotoxic effects (Menezes et al., 2013). Therefore, it is essential to evaluate the effect of cyanotoxins on human kidney cells (Piyathilaka et al., 2015). HEK293 cells were chosen for study because of their modest growth rate and the ability of these cells to readily express transfected mammalian proteins (Cheng et al., 200; Zhu et al., 1998). Human renal carcinoma line ACHN, is a good in vitro model of renal tubular epithelial cells (Taguchi et al. 1998).

The present study demonstrated that cyanotoxins could cause cytotoxic effects on kidney cells. MC-LR was the most toxic while MC-LW was least toxic cyanotoxin on both cell types tested. MC-RR, MC-LF and Nodularin had moderate cytotoxicity on human renal cells. Due to its higher toxicity of MC-LR, it is mandatory to study further about the toxicity and the underline mechanisms of its toxicity on human renal cells.

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