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A simple colorimetric method to detect biological evidence of human exposure to microcystins☆

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Abstract

Toxic cyanobacteria are contaminants of surface waters worldwide. Microcystins are some of the most commonly detected cyanotoxins. Biological evidence of human exposure may be difficult to obtain due to limitations associated with cost, laboratory capacity, analytic support, and expertise. We investigated the application of an enzyme-linked immunosorbant assay (ELISA) to detect microcystins in human serum. We analyzed ten serum samples collected from dialysis patients who were known to be exposed to a mixture of microcystins during a 1996 outbreak in Brazil. We applied a commercially available ELISA method to detect microcystins in serum, and we compared the ELISA results to a more specific method, liquid chromatography/mass spectrometry (LC/MS) that was also used to detect microcystins in serum. The Spearman correlation coefficient was calculated using serum microcystin concentrations in split samples obtained by the two methods. Serum microcystin concentrations were similar, and we found good correlation of microcystin concentrations between the two methods. The ELISA detected total microcystins, median = 19.9 ng/ml; LC/MS detected microcystin-LR equivalents, median = 21.2 ng/ml; Spearman $r = 0.96$, $p < 0.0001$. We found that ELISA is a simple, accessible method to screen human serum for evidence of microcystin exposure.

Keywords: Microcystins; Human exposure; Immunoassay; ELISA

1. Introduction

Microcystins (MCYST) are a group of cyclic polypeptide hepatotoxins of varying potency (Rinehart et al., 1994). They are produced by at least six genera of cyanobacteria, and MCYST occurrence has been reported worldwide. Surface waters may become contaminated with toxin-producing cyanobacteria and humans may be exposed to cyanotoxins, including MCYST, when such water is used for recreation or as a source of drinking water.

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Human health effects associated with exposure to cyanotoxins have been reported previously (Falconer et al., 1983; Jochimsen et al., 1998; Annadotter et al., 2001). Because toxic blooms may recur periodically in surface drinking and recreational water sources, people may be exposed to cyanotoxins in an episodic manner, yet documentation of evidence of human exposure is rare (Falconer et al., 1983; Annadotter et al., 2001; Maršálek et al., 2001; Carmichael et al., 2002).

Biological assessment of human exposure to cyanotoxins has historically required specialized laboratory equipment and expertise, limiting biological sample analysis to a few specialized analytic laboratories. Analysis of biological sample matrices pose challenging analytical problems: the concentration of MCYST is generally very low, typically at nanograms per milliliter or less, the matrix is complex, and MCYST is often bound by protein or even covalently conjugated to form another compound. Simple methods that can provide biological evidence of human MCYST exposure are needed for the successful implementation of epidemiologic and health studies.

Our goal was to determine if a commercially available enzyme-linked immunoflourescent antibody (ELISA) colorimetric assay could be used to screen human biological samples for evidence of MCYST exposure. We used serum from MCYST intoxicated humans with documented exposure to compare the sensitive, but relatively nonspecific ELISA against the more specific liquid chromatography/mass spectrometry (LC/MS) with electron spray ionization (ESI) method (Harada et al., 1999).

2. Materials and methods

2.1. Human biologic samples

A well-documented outbreak of human illness and death associated with MCYST toxicity occurred among dialysis patients during 1996 in Caruaru, Brazil. The circumstances of exposure and the health consequences of the patients' MCYST intoxication have been detailed in previous reports (Jochimsen et al., 1998; Carmichael et al., 2001). Brazilian health authorities and the personnel from US Centers for Disease Control and Prevention gathered human serum samples during and after this outbreak of MCYST intoxications. For the purpose of this methods comparison, we analyzed a subset of 10 serum samples derived from 10 exposed Caruaru dialysis patients.

2.2. Sample preparation

Archived serum samples collected from Caruaru patients during 1996 were thawed. Then 1-mL aliquots were transferred to 15-mL Corex glass tubes; 10 mL of methanol was added to each tube, mixed and centrifuged at 9000 rpm for 30 min. The supernatant was decanted into 20 mL scintillation vials. The pellet was resuspended with 5 mL of MeOH, centrifuged as before, and added to scintillation vials.

Five mL of hexane was then added to each vial. The vials were then capped and vortexed (Fisher Vortex Genie 2, Scientific Industries, Bohemia, NY). The hexane layer was discarded and the methanol layer was washed three more times with 5 mL hexane.

Samples were dried under vacuum in a Speed VAC Concentrator at 40 °C and then taken up in 2 mL of 5% HOAc. This solution was passed through an Oasis HLB solid phase extraction (SPE) cartridge (Waters, Milford, MA, USA). The HLB SPE cartridge was conditioned with 1 column volume of MeOH and 1 column volume of water.

The SPE cartridge was washed with 5 mL of 30% (v/v) MeOH in water. The MCYST fraction was then eluted with 5 mL of MeOH and dried. Samples were resuspended in 1 mL of 10% (v/v) MeOH in water and centrifuged at 10,200 rpm for 3.5 h through a YM-10 (Millipore, Bedford, MA) molecular weight cutoff filter.

These extracted serum samples were divided into equal volumes and analyzed for MCYST using an ELISA plate kit (EnviroLogix, Inc., Portland, ME, USA) and by LC/MS. Results were expressed as MCYST-LR equivalents. Toxin standards were extracted from *Microcystis aeruginosa* cultures maintained at Wright State University, and were purified to $>98\%$.

2.3. ELISA conditions and procedure

Direct competitive ELISA plate kits were used for detection and quantitation of free MCYST. They contain a polyclonal rabbit antibody raised against MCYST-LR conjugated to bovine serum albumin (BSA). The analyte competes with the MCYST enzyme conjugate (MCYST-LR peroxidase) for the antibody binding site. Addition of a substrate solution induces a color change, which can be read at 450 nm using a plate reader. Dark colors indicate lower amounts of MCYST and light colors indicate higher toxin concentrations. Our calibration curve used concentrations between 0.5 and 50 ng/mL. The limit of detection (LOD) for the assay is 0.147 ppb (ng/mL). All reagents and standards for the assay were supplied in the Envirologix plate kit.

All serum samples (in 5% MeOH) and standards were run in triplicate. Briefly, 125 μL of assay diluent was added to each well. We added 20 μL of negative control, 20 μL of each calibrator and 20 μL of sample to their respective wells. The contents were mixed with a circular motion for 20–30 s, the plate covered with Parafilm (Structure Probe, Inc./SPI Supplies, West Chester, PA, USA) and incubated at room temperature for 30 min. We then added 100 μL of MCYST-enzyme conjugate to each well. We mixed the contents, covered with Parafilm and incubated at room temperature for 30 min. We flooded the contents of the plate with PBS wash solution four times then would shake to empty each time. We used a Dynatech AM60 multi-reagent