Chapter 30: Human Health Effects Workgroup Poster Abstracts

Serologic evaluation of human microcystin exposure

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Introduction

Microcystins (MCYST) are among the most commonly detected toxins associated with cyanobacteria blooms worldwide. Biological evidence of human exposure is needed in order to evaluate potential MCYST-associated health effects. MCYST are detectable in free and bound forms in human serum. We will provide an overview of selected methods to detect biological evidence of exposure in humans, and will identify some uncertainties associated with interpretation of results.

Methods

We analyzed serum samples collected from MCYST-exposed patients after exposure events at Brazilian dialysis clinics during 1996 and 2001. We used a commercially available enzyme linked immunoassay (ELISA) method to detect free MCYST, liquid chromatography/mass spectrometry (LC/MS) to detect free MCYST, and gas chromatography/mass spectrometry (GC/MS) to detect 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB). MMPB is derived from both free and protein-bound MCYST by chemical oxidation, so it appears to represent total MCYST present in serum.

Results

Exposed patients provided blood samples for analysis after exposure. In a subset of 10 serum samples we found similar concentrations of free MCYST between the ELISA and LC/MS methods (Spearman $r=0.96$, $p<0.0001$). ELISA measurement of free MCYST was consistently lower than MMPB quantification of total MCYST. ELISA measured free MCYST as 8 – 51 % of total MCYST. Among the larger exposed population, we found evidence of free MCYST in patient serum for more than 50 days after the last date that documented MCYST exposure occurred.

Conclusion

MCYST are present in serum in free and protein-bound forms, though the nature of protein bound forms is uncertain. Analysis of serum samples for the presence of free MCYST may be performed in a cost-effective manner using screening assays such as the ELISA, but they underestimate total circulating concentrations. The relationship between free or total MCYST and absorbed dose is unknown due to limited knowledge of distribution and clearance. We found that free MCYST concentrations in patient serum may be detected for more than 50 days after the last documented exposure occurred. However, it is possible that patients experienced continued MCYST exposure by some route that was undetected during this study. Research is urgently needed to elucidate the human toxicokinetics of MCYST, in part to determine how measured serum levels can be used to estimate MCYST exposure.

Characterization of chronic human illness associated with exposure to cyanobacterial harmful algal blooms predominated by *Microcystis*

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Introduction

Health effects from exposure to surface waters in the USA experiencing blooms of toxigenic cyanobacteria have not been well characterized. We initially evaluated seven cases of chronic illness following exposure to Lake Griffin, a member of the St. John's chain of lakes in Florida, during a bloom of *Microcystis* that was reported by the St. John's Water Management District. All seven people complained of multiple–system symptoms and demonstrated deficits in visual contrast sensitivity (VCS). Differential diagnoses based on medical histories, physical examinations, complete blood counts, comprehensive metabolic profiles, and assessments of both potentially confounding factors and toxic exposures indicated that exposure to the *Microcystis* bloom was the likely cause of illness. Patient reevaluations after 2 weeks of cholestyramine (CSM) therapy to bind and eliminate toxins demonstrated a statistically significant decrease in the number of symptoms and increase in VCS. The evidence indicated that exposure to the *Microcystis* bloom caused a biotoxin–associated illness similar to those previously reported in association with exposures to waters with high levels of toxigenic dinoflagellates and with exposures to water–damaged indoor environments exhibiting microbial amplification, including toxigenic fungi. We currently report a cohort of 10 patients exposed to *Microcystis* blooms who were evaluated before and after CSM therapy.

Hypotheses

Exposures to *Microcystis* blooms are associated with: (1) chronic illness characterized by multiple–system symptoms and VCS deficits; 2) increased blood

levels of leptin and MMP9; 3) decreased blood levels of aMSH, ADH/osmolality, VEGF and ACTH/cortisol, and; 4) symptom resolution, and normalization of VCS and all biomarkers following CSM therapy.

Methods

Ten cases of chronic illness following exposure to *Microcystis* blooms were evaluated using the methods described above. Exposures to *Microcystis* blooms were determined to be the likely cause of illness. Three cases were exposures to blooms predominated by M*icrocystis* and reported by the St. John's Water Management District, whereas six cases were exposed to *Microcystis* blooms reported by the Maryland Department of Natural Resources. The number of symptoms, VCS, and blood levels of leptin, cortisol, osmolality, MMP9, VEGF, aMSH, and ACTH, were measured before and after CSM therapy, and HLA DR genotypes were identified. Repeated measurements of C3a, C4a, interleukin–10, and interferon alpha were also obtained from 3 cases. All measures were compared to those previously obtained from 239 unexposed well patients.

Results

The mean number of symptoms reported by patients was 19.7 out of 37 assessed before CSM therapy, and 3.2 following therapy. VCS increased by about 40% after therapy. Blood levels of blood levels of leptin and MMP9 were significantly higher than controls prior to therapy, whereas aMSH, ADH/osmolality, VEGF and ACTH/cortisol were low. All biomarkers normalized after 2 weeks of therapy except for aMSH. Two HLA DR haplotypes were significantly overrepresented in the cohort. All three cases for whom C3a, C4a, interleukin–10, and interferon alpha were measured showed elevated levels prior to therapy and normal levels following therapy.

Conclusion

The evidence indicated that exposures to *Microcystis* blooms may cause a form of chronic, biotoxin–associated illness that is characterized by abnormalities in symptoms, VCS and multiple biomarkers that resolves with CSM therapy. A randomized, double–blind, placebo–controlled, clinical trial and methods to measure cyanotoxins in blood is needed to confirm this hypothesis.