Human intoxication by microcystins during renal dialysis treatment in Caruaru—Brazil

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Abstract

In February 1996, an outbreak of illness occurred at a hemodialysis clinic in Caruaru, Pernambuco State—Brazil. At this clinic 116 (89%) of 131 patients experienced visual disturbances, nausea, vomiting, and muscle weakness following routine haemodialysis treatment. Subsequently, 100 patients developed acute liver failure. As of December 1996, 52 of the deaths could be attributed to a common syndrome now called ‘Caruaru Syndrome’. Examination of previous years’ phytoplankton counts showed that cyanobacteria were dominant in the water supply reservoir since 1990. Analyses of carbon and other resins from the clinic’s water treatment system plus serum and liver tissue of patients led to the identification of two groups of hepatotoxic cyanotoxins: microcystins (cyclic heptapeptides) in all of these samples and cylindrospermopsin (alkaloid hepatotoxic) in the carbon and resins. Comparison of victims symptoms and pathology with animal studies on these two cyanotoxins, leads us to conclude that the major contributing factor to death of the dialysis patients was intravenous exposure to microcystins, specifically microcystin-YR, -LR and -AR. In 2000, a review of the Brazilian regulation for drinking water quality, promoted by Brazilian Health Ministry with collaboration of PAHO, incorporated cyanobacteria and cyanotoxins into this new regulation as parameters that must to be monitored for water quality control.

Keywords: Cyanobacteria; Microcystins; Cylindrospermopsin; Human health

1. Introduction

Increasing eutrophication of fresh and coastal water bodies has been produced by anthropogenic nutrient and other pollutant enrichment. It has become more widespread mainly in some regions where the growth of agricultural industry and urbanization has undergone rapid increase. These processes have not been followed by an improvement in waste water treatment. One of the main problems related to human health and water
quality in eutrophic waters is the proliferation of cyanobacterial blooms.

Planktonic cyanobacteria are a natural component of phytoplankton in most surface waters of the world. However, high cyanobacterial biomass contributes to aesthetic problems, impairs recreational use and has been implicated in the development of obnoxious taste and odor in water supplies. In addition to these deleterious effects, freshwater cyanobacteria have been receiving increased attention due to their ability to produce toxins. (Carmichael, 1997; Chorus and Bartram, 1999; Falconer, 1999).

Acute hepatotoxicosis associated with hepatotoxins is the most commonly encountered toxicosis involving cyanobacteria. The most studied hepatotoxins produced by cyanobacteria are the cyclic heptapeptides called microcystins (Carmichael, 1997). These cyanobacterial hepatotoxins cause deaths by liver hemorrhage within a few hours of an acute dose. The mammalian toxicity of microcystins is due to inhibition of protein phosphates enzymes 1 and 2A (Falconer, 1999; Mackintosh et al., 1990; Runnegar and Falconer, 1986).

Animal intoxication by consumption of water contaminated with toxic cyanobacteria have been well documented around the world (Chorus and Bartram, 1999). Human deaths by oral consumption of cyanotoxins have been suspected but not confirmed mainly due to the difficulty in obtaining good data regarding vectors and circumstances that would confirm the presence of cyanotoxins in human food or water supplies. However, oral consumption is not a unique route of exposure for humans. The first documented human deaths from cyanobacterial hepatotoxins occurred following intravenous exposure in a dialysis clinic in Caruaru city, Brazil, during 1996 (Jochimsen et al., 1998; Carmichael et al., 2001).

In February 1996, 116 (89%) of 131 patients receiving routine renal dialysis treatment at clinic A began to complain of headache, eye pain, blurred vision, nausea and vomiting. The first death occurred on February 20. Subsequently, 100 patients developed acute liver failure, and 52 had died by December of 1996 with a common syndrome now called as ‘Caruaru Syndrome’ (Barreto et al., 1996; Jochimsen et al., 1998).

According to the reports from Pernambuco state health authorities and confirmed by the epidemiological investigation (Jochimsen et al., 1998), the referral clinic was not receiving reticulated water directly from the municipal water-treatment plant, during the 1996 summer drought. Instead, this clinic received water without complete treatment, distributed by tank trucks from the municipal water-treatment plant. Occasionally, the truck driver was directed to add chlorine to the water in his truck when the visual turbidity of water was high. After arriving at the clinic, water was treated in its in-house water treatment system; a process that involved sand, carbon, and cation/anion exchange filtrations, followed by a micropore filtration, before being used for hemodialysis. Dialysis was administrated by a conventional hemodialysis system: reverse osmosis was not used in the water treatment process.

The previous knowledge about cyanobacterial bloom occurrence in Brazilian northeastern reservoirs, the chemical stability of cyanobacterial hepatotoxins in the water and the similarity of the symptoms described for the patients with those already observed from animals subject to cyanobacterial hepatotoxicoses, made us to suspect that those human intoxications could be related to cyanobacterial hepatotoxins. In order to test this hypothesis, an examination of phytoplanktonic species from water sources, analysis of cyanotoxins in clinic’s water treatment system, plus serum and liver tissue from the patients were done. This report summarizes the results of that investigation and provides a brief comment on the potential human health risk from consumption of water contaminated with toxic cyanobacteria.

2. Material and methods

Phytoplankton samples from Tabocas reservoir (water supply for Caruaru city) were collected at the end of March 96 and analyzed by semi-quantitative methods, using an inverted microscope. Previous phytoplankton data from this reservoir and from treated water as well as phytoplankton data in raw and treated water from March 96 until April 96 were provided by
the State of Pernambuco Company for Sanitation (COMPESA).

Samples of carbon, sand, cationic and anionic resins, used in the dialysis clinic in-house water system filters, were extracted three times with methanol 100%. Extracts were dried and resuspended in distilled water passed through a C-18 cartridge that was washed and eluted with H₂O, methanol-20%, and methanol-100%. The methanol-100% fractions were dried, resuspended in 1.0 ml of 1:1 methanol/deionized water and analyzed by an enzyme-linked immunosorbant assay (ELISA) using a polyclonal antibody against microcystins.

Blood sera (17 samples from 12 victims), liver (52 samples from 39 victims) and from control patients (five liver and 12 sera samples) were provide by state health authorities from Pernambuco and sent though the Centers for Disease Control (CDC), in Atlanta, Georgia USA. These samples were extract and analyzed by ELISA and chemical methods (HPLC/PDA, MALDI/TOF and ESI/MS) as described in Carmichael et al. (2001). Further analysis for cylindrospermopsins (alkaloid hepatotoxin) in sand, carbon, ion exchange resins and liver and sera samples were done by chemical methods (HPLC/MS/MS), also described in Carmichael et al. (2001).

3. Results

The analysis of phytoplankton from Tabocas reservoir demonstrated that cyanobacterial species were dominant, representing 99% of the total density of phytoplanktonic community. The most common cyanobacteria present at the end of March were: *Aphanizomenon manguinii* and two species of *Oscillatoria*. The number of cyanobacterial organisms in the raw water before water treatment was 20,882/ml. Examination of phytoplankton data showed that cyanobacteria species had dominated the reservoir since 1990 (Fig. 1). The most common genera were *Microcystis*, *Anabaena* and *Cylindrospermopsis*. *Microcystis* and *Anabaena* are known as microcystins producers while *Cylindrospermopsis* can produce cylindrospermopsin. All these genera have been responsible for cyanobacterial blooms in various Brazilian reservoirs (Azevedo et al., 1994; Bouvy et al., 2000; Magalhães et al., 2001; Yunes et al., 1996). However, as phytoplankton counts were not being made at the time of the outbreak, toxic cyanobacteria species during the period of intoxication could not be identified.

![Fig. 1. Quantitative analysis of phytoplankton in raw water (A and C) from Tabocas reservoir and in treated water (B and D) distributed by Caruaru city water treatment plant. (A and B) Data base from 1990 to 1995; (C and D) data base from March to April 1996.](image-url)
Analysis of carbon, sand and cation/anion resin filters showed microcystin in the μg/g range (0.5–2.1). Further examination of methanolic extracts from these material provided chemical evidence for cylindrospermopsin contamination, mainly in the carbon extract (19.7 μg/g).

All victim sera and liver tissue were positive for microcystins. The average microcystin concentration in 52 liver samples from 39 victims who died from February to December of 1996 was 223 ng/g (Fig. 2). Chemical analysis of some liver samples identifies microcystins-YR, -LR and -AR. However, chemical analysis of these samples for cylindrospermopsin did not confirm the presence of this molecule (Carmichael et al., 2001).

According to Jochimsen et al. (1998), histopathological examination by light microscopy of liver tissue obtained from 16 victims revealed an uniform pattern with disruption of liver plates, liver cell deformity, necrosis, apoptosis, cholestasis, cytoplasmic vacuolization, mixed leukocyte infiltration and multinucleated hepatocytes. This pathological picture is very similar to that found with previous laboratory animal experiments involving microcystin exposure (Fig. 3) (Dabholkar and Carmichael, 1987; Runnegar et al., 1987; Theiss et al., 1988).

An estimated microcystin concentration of 19.5 μg/l of water used during dialysis treatment from 13 to 17 February 1996 was suggested by Carmichael et al. (2001). This value was calculated by taking an average of 1500 g for liver weight and the amount of water used during a dialysis treatment—120 l. Two correction factors was used: (1) the amount of microcystins in the liver as a percentage of the total exposure—estimated as 50%; (2) the amount of microcystins covalently bound to protein phosphatases 1 and 2A that might not be recognized by the ELISA assay—estimated as 80%. This estimated value is approximately 20 times higher than concentration of 1 μg/l/day, the WHO limit for safe levels of oral consumption of microcystins in drinking water.

Fig. 2. Average of microcystin concentration (ng/g) in liver samples from Caruaru dialysis victims per month of death. Data represent 52 livers samples from 39 victims, covering February to December 1996. The average of microcystins concentration in sera from affected patients was 2.2 ng/ml. ◯ patient deaths; ■ microcystins concentration.
4. Discussion

The available biological and chemical evidence is consistent with microcystins from Tabocas reservoir water being a major factor in the deaths of dialysis patients. The results suggest that the intoxication of patients could have been caused by microcystins release from cyanobacterial cells that were lysed by chlorination in the tank trucks. If we consider the amount (120 l) of water necessary for each individual hemodialysis and the high density of cyanobacterial cells in the water observed in March, it allow us to postulate that in February the cyanobacterial concentration would have been sufficient to produce a microcystin concentration capable of causing this acute human intoxication.

There are many important public health lessons and much scientific information to be gleaned from this first confirmed outbreak of human poisoning involving cyanotoxins. Since increasing levels of nutrients are being found in reservoirs and natural water supplies, it is highly probable that another episode of cyanotoxin poisoning will occur unless measures are taken to understand the role of these toxins in water-based disease. There should be programs of watershed management to

![Fig. 3. Microcystin effects on human and rat liver. Light micrographs of normal rat liver (A) and human liver (B) and a liver sample from a Caruaru victim (C) compared with rat liver affected by microcystin (D). Liver plates disruption and cell deformity are very similar between laboratory animal experiments and affected dialysis patient.](image-url)
reduce nutrient inputs, cyanotoxin monitoring programs to alert authorities to the presence of cyanotoxins and improvements in water treatment techniques to reduce or remove cyanotoxins from finished water supplies.

Guideline to control water quality used for dialysis have not considered cyanotoxins as one parameter that need to be monitored. However, a conventional water treatment plant, using flocculation, precipitation, sand filtration and chlorination is not enough to remove cyanobacterial toxins from water supplies with high levels of eutrophication and cyanobacterial dominance. Dialysis centers need to consider the potential for cyanobacterial toxicity from the water piped from public water plants. A rigorous control of in-house water treatment system at dialysis clinics also needs to be followed. Last year the Brazilian Health Ministry with collaboration of the PAHO, considering the necessity for better control of water quality, promoted a review of the Brazilian regulations for drinking water quality. Cyanobacteria and cyanotoxins were incorporated into this new regulation as parameters that must to be monitored.

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References


