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Comparative Toxicity of the Cyanobacterial Toxin Cylindrospermopsin Between Mice and Cattle: Human Implications

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Abstract

The cyanobacterial toxin cylindrospermopsin is produced by *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* in many parts of the world. A human poisoning incident occurring at Palm Island, Queensland, Australia in 1979 was subsequently ascribed to cylindrospermopsin. The structure of cylindrospermopsin, a tricyclic guanidinium moiety bridged to hydroxymethyluracil, was deduced in 1992. A number of studies have investigated the acute toxicity of cylindrospermopsin in mice. It is primarily a hepatotoxin with a 24-hour acute intraperitoneal (IP) LD_{50} of 2 mg/kg, a 5-day acute i.p. LD_{50} of 0.2 mg/kg and a 5-day acute oral LD_{50} of approximately 6 mg/kg. A human health risk assessment using data from longer-term oral dosing studies suggests a guideline value for cylindrospermopsin in drinking water of approximately 10 µg/L. We have recently studied cattle poisonings by cylindrospermopsin and detected the toxin in a number of tissues after necropsy. Concentrations of 1 mg/L or above in drinking water (dose is approximately 50 µg/kg/day) were shown to result in cattle death after short-term exposure (less than 10 days). Oral dosing of mice at levels up to 5 mg/L with cylindrospermopsin in drinking water for 90 days did not produce any significant toxicity. Human health risk assessment based on cattle however, which are much more sensitive to cylindrospermopsin than rodents, would produce a guideline for human drinking water of approximately 0.05 µg/L. A consideration of reported human poisoning incidents that implicate cylindrospermopsin suggests that humans may also be more sensitive than rodents to this toxin.

Introduction

The cyanobacterial toxin cylindrospermopsin was discovered as a result of an acute human poisoning incident in Palm Island, North Queensland, Australia, in 1979. The epidemic lasted 21 days and reached its peak by the eighth day (Byth, 1980). The illness was hepatoenteric in nature and commenced after Solomon Dam, the water supply for the island, was treated with copper sulfate to control a dense cyanobacterial bloom. An epidemiological study showed that only persons utilising the reticulated water supply became ill (Bourke *et al.,* 1983). From all available evidence, Bourke *et al.* (1983) and Hawkins *et al.* (1985) retrospectively postulated that the sickness was related to cyanobacterial toxicity. The cyanobacterium, *Cylindrospermopsis raciborskii* was believed to be the causative agent and was shown by Hawkins *et al.* (1985) to produce severe toxicity to the liver and other organs of intraperitoneally dosed experimental animals. The toxin cylindrospermopsin was later isolated and structurally identified in material from cultures of this organism (Ohtani *et al.,* 1992). Chemically this toxin is a tricyclic guanidium moiety bridged to hydroxymethyl uracil. The toxin is very zwitterionic and therefore very hydrophilic, which has implications for its distribution in drinking water reservoirs. Cylindrospermopsin has also been suggested as the cause for an illness termed "Barcoo fever" that was widespread in Northern outback Australia (Hayman, 1992). The symptoms described for Barcoo fever were very similar to those reported in the Palm Island community during the poisoning incident in 1979. More recently, cylindrospermopsin has been found in addition to microcystins in water filters in the dialysis center in Caurary, Brazil where 76 patients died of acute liver disease in 1996 (Carmichael *et al.,* 2001).

To date, acute and sub-chronic data for cylindrospermopsin toxicity in mice have been produced (Hawkins *et al.,* 1985; Ohtani *et al.,* 1992; Terao *et al.,* 1994; Falconer *et al.,* 1999a; Seawright *et al.,* 1999; Shaw *et al.,* 2001). Recently cattle mortalities have been attributed to cylindrospermopsin that was present in farm water storages featuring heavy blooms of *C. raciborskii* (Saker *et al.,* 1999; McKenzie *et al.,* 2003). To date, however, no evaluation of the comparative toxicity of cylindrospermopsin in mice and cattle has been published. In this study we have examined the death of cattle from two separate poisoning incidents involving cylindrospermopsin, have confirmed the presence of this toxin in drinking water and cattle tissues, and have histologically shown the pathology of intoxication to be typical of cylindrospermopsin poisoning in experimental animals.

Methods

Two cattle-poisoning incidents were investigated as reported in McKenzie *et al.* (2003). Case A involved the deaths of 10 cattle in Central Queensland in August 2001 and case B involved 45 cattle in Northwest Queensland in October 2001. Cylindrospermopsin in water samples from farm water supplies was analyzed using HPLC-MS/MS according to the method of Eaglesham *et al.* (1999). Toxin concentration in cattle rumen contents was determined by centrifuging to remove particulate matter and then diluting before HPLC-MS/MS analysis as for water. Liver, kidney and muscle tissue was macerated with 75%

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methanol/water and sonicated. The supernatant was removed, partitioned with hexane to remove lipid material and the aqueous layer was analyzed for cylindrospermopsin content using HPLC-MS/MS as above. Histopathology was performed on cattle organs by fixing tissues with 10% formalin and processing by standard techniques with hematoxylin and eosin staining.

Results and Discussion

Affected cattle were ill (clinical signs were lethargy and recumbency) for 3–4 days before dying. Necropsy revealed typical cylindrospermopsin toxicity with pale mottled livers and distended gall bladders. Histology revealed hepatocyte degeneration and necrosis, nephrosis and multifocal cardiac muscle degeneration. Cylindrospermopsin concentrations in water and tissues are given in Table 1.

The results demonstrate that water with a cylindrospermopsin concentration as low as approximately 1000 µg/L may be fatal to cattle. The rumen contents in case A contained cylindrospermopsin at roughly half the concentration found in the drinking water. If this relationship holds, the toxin concentration in drinking water in case B would be about 10,000 µg/L. The liver and kidney also had detectable concentrations of cylindrospermopsin. These organs are the main targets for this toxin and distribution studies using ^{14}C-labelled cylindrospermopsin (Norris *et al.,* 2001) demonstrated that they are the preferential sites for toxin accumulation. Unlike the microcystins which are transported into the liver through the bile acid transport mechanism, no active uptake mechanism appears to operate with cylindrospermopsin and uptake via passive diffusion has been suggested (Chong *et al.,* 2002). The lack of cylindrospermopsin in edible muscle of fatally poisoned cattle is of note in human health risk assessment of exposure to this toxin in meat. No international guidelines have been developed for cylindrospermopsin in drinking water to date. Using mouse data, however, it is possible to suggest human drinking water guidelines using World Health Organization protocols as described by Falconer *et al.* (1999b). The acute oral LD_{50} for mice is approximately 6000 µg/kg (Seawright *et al.,* 1999). Our data from a range of mouse-dosing studies have been reported (Shaw *et al.,* 2001), and guideline values of 1.75 µg/L were calculated from 14-day oral gavage studies. Values of 7.0 µg/L and 10.5 µg/L were calculated from the results of 28-day repetitive oral drinking water and 90-day oral drinking water studies, respectively. Due to its longer duration, the 90-day drinking water study is considered the most accurate. The no observable adverse effect level (NOAEL) in this study was 150 µg/kg/day. The results of an 11-week study involving daily oral gavage dosing of mice with cylindrospermopsin have been reported (Falconer and Humpage, 2002). That study suggests a guideline value for cylindrospermopsin in drinking water of 1 µg/L.

In the case of the cattle poisonings, the lowest concentration in drinking water that was fatal in the short-term (less than 7 days) was 1050 µg/L. Derivation of approximate guidelines for cylindrospermopsin in drinking water for humans derived from the cattle data is presented below:

- For cattle, lethal dose per day = [cylindrospermopsin] × volume water consumed / body weight
- Lethal dose = 1050 µg/L × 10L / 250 kg = 42 µg/kg/day.

Assuming a similar ratio between lethal dose and NOAEL for mice and cattle, then:

- $NOAEL_{cattle} = NOAEL_{mice} \times \text{lethal dose}_{cattle} / \text{lethal dose}_{mice}$
- $NOAEL_{cattle}$ = 150 µg/kg/day × 42 µg/kg/day / 6000 µg/kg/day = 1.05 µg/kg/day.
- Tolerable daily intake (TDI) = NOAEL / 1000 (uncertainty factors) for interspecies and intraspecies variations and less than lifetime exposure. Therefore TDI = 1.05 µg/kg/day / 1000 = 0.00105 µg/kg/day.
- Guideline value (GV) = TDI × body weight × proportion of toxin from water / daily water intake
- GV = 0.00105 µg/kg/day × 70 kg × 1 / 2L = 0.037 µg/L

The guideline value for cylindrospermopsin in human drinking water calculated from cattle data is orders of magnitude lower than that calculated from mouse data. The question remains as to whether human sensitivity to this toxin is more similar to cattle or mice. Considering the Palm Island human poisoning incident, if this was due to cylindrospermopsin toxicosis, it was an acute poisoning incident with severe symptoms requiring hospitalization. In our experience it is highly unlikely that a large drinking water reservoir would contain cylindrospermopsin at concentrations exceeding 1000 µg/L. At this concentration, the daily dose for humans would be approximately 30 µg/kg/day, which is less than the NOAEL for mice but potentially lethal to cattle. It appears that the mouse model may seri-

Table 1 Cylindrospermopsin concentrations in drinking water and cattle tissues and organs from poisoning cases.

	Cylindrospermopsin concentration*	
Sample type	Case A	Case B
Water	1050	Not analyzed
Rumen contents	570	5700
Liver	7.4–51	Not analyzed
Kidney	9.4–29	Not analyzed
Skeletal muscle	Not detected at an LOD of 0.2	Not analyzed

*Concentration in micrograms/litre for water and rumen contents and micrograms/kilogram for tissues and organs.

ously underestimate the true toxicity of cylindrospermopsin to humans and it is suggested that studies involving another animal model in place of rodents be undertaken before guideline values for cylindrospermopsin in human drinking water are established.

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