Chapter 27: Health effects associated with controlled exposures to cyanobacterial toxins

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Abstract

The cyanobacterial toxins of concern as potential human health hazards are those known to occur widely in drinking water sources, and therefore may be present in water for human use. The toxins include a diverse range of chemical compounds, with equally diverse toxic effects. These toxins are not limited to individual cyanobacterial species or genera, and all of the toxins of concern to human health are produced by multiple cyanobacterial species.

Microcystins

The acute effects of microcystins have been investigated mainly by intraperitoneal (i.p.) dosing or oral dosing of experimental animals. Limited information is available from inhalation or intranasal dosing of rodents. No controlled dosing trials have been undertaken with human subjects.

It is well established that the main target for toxic effects of microcystins in mammals is the liver, with adverse effects also seen in the small intestine and kidney. In large animals hemorrhagic responses have also been seen after oral dosing of toxic extracts. Hepatotoxicity is a primary response because microcystins are transported actively into hepatocytes via organic anion transporters which concentrate the toxin in target cells. The toxicity of microcystins results from the inhibition of the catalytic

subunit of protein phosphatases 1 and 2A. These enzymes play a vital role in intracellular regulation, in balancing the phosphorylation and dephosphorylation of regulatory and structural proteins, hence altering enzyme activities and structural integrity of the hepatocyte. As a consequence hepatocytes are highly sensitive to microcystin toxicity; the concentration of microcystin causing 50% rat hepatocyte deformity during *in vitro* incubation for 30 min is 30nM. This initial rapid response of hepatocytes to microcystin exposure is cell deformation caused by disruption of the cytoskeleton through hyperphosphorylation of intermediate filament proteins. *In vivo* this results in disintegration of the hepatic cellular architecture, with extensive bleeding into the liver resulting in rapid death of the animal. The i.p. LD_{50} over 24h for rodents for most microcystin variants is 50-100μg/kg bodyweight. The microcystin variants having two arginine residues in the molecule (MCYST-RR variants) have lower toxicity, with an approximate LD_{50} of 300μg/kg. The earliest time-to–death after a lethal intraperitoneal dose in mice is approximately 20-30 minutes. Enterocytes lining the small intestine also actively take up microcystins, and show the same deformation responses *in vitro* to microcystins as do hepatocytes. This damage to enterocytes is likely to be the cause of some of the observed gastrointestinal responses to oral microcystin poisoning.

Intranasal exposure to microcystin-LR is equally toxic as i.p. exposure, with extensive damage to nasal mucosa. Oral toxicity is 5-10 fold lower than i.p. toxicity, and in mice is affected by age, with older mice being more sensitive. Male mice are appreciably more sensitive than female mice, which require at least a two-fold higher microcystin dose for the same adverse response. Time-to-death from a lethal oral dose in rodents is variable, around 6-20 hours. In sheep acute lethal effects are seen from 18h after intra-ruminal dosing, with major liver damage.

Oral sub-chronic dosing trials with microcystins have been undertaken in mice and pigs, with the aim of determining a maximum No Observed Adverse Effect Level (NOAEL). In mice this was carried out by gavage with pure microcystin-LR, and a NOAEL for male mice was determined at 40μg/kg/day, based primarily on histopathological evidence of liver injury and serum analysis for liver function enzymes. In pigs the microcystins supplied though the drinking water were a thoroughly characterised *Microcystis aeruginosa* extract containing several microcystins. This trial resulted in determination of a Lowest Observed Adverse Effect Level (LOAEL) of 100μg/kg/day, also by histopathological examination of the liver. Chronic oral toxicity trials in mice using extracts of *Microcystis* supplied in the drinking water for upto one year showed increased general mortality from infections, and chronic active liver injury at higher doses.

Exposure to drinking water containing *Microcystis* extracts gave evidence of increased tumours in these mice.

Laboratory investigation of tumour promotion in rodents by microcystin has given strong evidence of non-phorbol ester type promotion. *In vivo* exposure to microcystin-LR has shown increased foci of pre-neoplastic cells in livers of mice initiated with carcinogen. Similarly supply of microcystin in drinking water to mice given azoxymethane as a colon tumour initiator, showed increased growth of hyperplastic colon crypts. Mice treated on the skin with the carcinogen dimethylbenzanthracine showed marked increases in papilloma growth when given *Microcystis* extract in drinking water.

The genotoxic effects of microcystin are still under discussion, with evidence from different investigations of both genetic damage and of lack of genotoxicity. There is also evidence of liver carcinogenesis by high repeated intraperitoneal doses of microcystin in mice. There are no non-rodent long term studies of chronic toxicity or carcinogenicity at present.

Reproductive toxicity studies have given conflicting results, possibly due to toxic mixtures of *Microcystis* extracts being injected intraperitoneally in mice. In studies using pure microcystin-LR, or by oral exposure of mice to microcystins in extracts, no clear reproductive or developmental injuries were reported.

Human toxicity is relevant to this discussion, though all evidence of human injury from microcystins is necessarily obtained from retrospective investigation with actual exposures not readily quantified. An epidemiological study of human liver function in populations exposed and unexposed to drinking water drawn from a reservoir carrying a large *Microcystis* bloom (treated with copper sulphate) showed a response characteristic of hepatotoxicity only in the exposed population, and only at the time of the *Microcystis* bloom. In a Brazilian clinic more than 50 dialysis patients died after perfusion with water containing the cyanobacterial toxins microcystin and cylindrospermopsin, with clear evidence of major liver damage.

In southern China there are areas of very high rates of primary liver cancer, and epidemiological studies have linked this to drinking of surface water containing microcystins, as well as to aflatoxin in the diet and infection by viral hepatitis. In another area in China, colon cancer rates have shown a correlation with concentration of microcystins in the various water sources.

Recent evaluation of carcinogenesis from microcystin exposure by the International Agency for Research in Cancer has determined that microcystin-LR and other microcystins are **possible carcinogens**, based substantially on their demonstrable tumour promoting effects in laboratory studies with rodents.

Cylindrospermopsins

These alkaloids have been investigated by i.p. and oral dosing of rodents, but not yet of larger mammals due to limited supply and high cost of the toxins. They are general cytotoxins, causing damage to liver, kidneys, gastrointestinal tract, endocrine organs, the immune system, vascular system and muscle. There appear to be two toxic responses, the rapid toxicity probably linked to a toxic metabolite formed by oxidation in the liver, as there is protection from toxicity by some cyt-P450 inhibitors. The other slower toxic response is due to protein synthesis inhibition, which is a longer-term toxicity showing much higher sensitivity to cylindrospermopsin. As a result, the i.p. LD_{50} in mice at 24h was 2,100 μg/kg bodyweight and the LD_{50} at 5-6 days was 200μg/kg. The acute oral LD_{50} is not yet clearly established in experimental animals, but appears to be about 5,000μg/kg.

Sub-chronic oral toxicity trials by gavage with purified toxin determined a NOAEL in male mice of 30μg/kg/day. This was based on adverse effects on kidney function, which appeared more sensitive than liver injury to cylindrospermopsin toxicity. A long-term oral toxicity study in male and female mice which used hematology and erythrocyte morphology as the most sensitive indicators of adverse effects, determined a Lowest Observed Adverse Effect Level (LOAEL) of 20μg/kg/day in both sexes of mice. Derivation of a provisional Guideline Value for cylindrospermopsin in drinking water from these data resulted in values of approximately 1μg/l., using the standard safety factors for subchronic toxicity in rodents.

In-vitro genotoxicity and mutagenicity testing of cylindrospermopsin in hepatocytes, lymphocytes and several transformed cell lines has demonstrated that it was genotoxic and mutagenic.

Preliminary results for oral carcinogenicity in mice indicate that it may be carcinogenic in a range of tissues.

Data are lacking for the detailed mechanism of action of cylindrospermopsin, though it has been shown that protein synthesis inhibition is exerted at the ribosome level. This is presently under investigation. Current unpublished studies of teratogenicity and developmental toxicity should be available shortly. Chronic oral carcinogenicity studies on cylindrospermopsin are urgently needed for both rodents and non-rodent species.

A human population on an island were exposed to drinking water drawn from a reservoir carrying a heavy bloom of *Cylindrospermopsis,* suffered acute hepato/gastroenteritis after the reservoir was treated with copper sulphate. About 150 children and adults were treated in the hospital, with 85 cases considered severe enough to be airlifted to a more ad-

vanced hospital with intensive care facilities. None died. Cylindrospermopsin was subsequently isolated from cultured *Cylindrospermopsis raciborskii* obtained from this reservoir.

Anatoxins

Anatoxin-a and homo-anatoxin-a are small neurotoxic alkaloids, which act as agonists at the neuromuscular junction, causing spontaneous firing and eventually death by respiratory failure. The acute i.p. LD_{50} is 375μg/kg in mice, the intravenous (i.v.) LD_{50} is less than 100μg/kg, with an intranasal LD_{50} of 2000μg/kg and no lethality observed at a 5000μg/kg oral dose. Repeated i.p. injection did not elicit resistance to toxicity. Data are available for sub-chronic oral toxicity. Anatoxin-a by gavage at 15,000μg/kg killed mice within 3 minutes, however 3 of 4 mice receiving 7,500μg/kg/day for 4 weeks survived with no post-mortem pathological changes. Nineteen of 20 mice receiving 3,000μg/kg/day for 4 weeks showed no effects. There is no evidence for reproductive, teratogenic or carcinogenic effects of anatoxin-a.

Anatoxin-a(s) is an organophosphate anticholinesterase causing salivation, muscle weakness, convulsions, and death by respiratory paralysis. The i.p. LD_{50} is 20μg/kg in mice, there are no oral toxicity data.

Dog deaths due to consumption of anatoxin-a and homo-anatoxin-a from cyanobacterial sources have been reported from several countries.

Though a recent accidental death of a teenager in the USA was attributed to consumption of anatoxin-a during swimming, there is doubt over the analytical results. No population-level adverse health effects have been reported.

Saxitoxins

These alkaloids block sodium channels in nerve axons, causing loss of sensation and paralysis and are highly toxic. Saxitoxins are best known as paralytic shellfish poisons, which have caused many human fatalities. The i.p. LD_{50} in mice is 8-10μg/kg, the i.v. LD_{50} is 3.4μg/kg and the oral LD_{50} is 260μg/kg for saxitoxin. Other saxitoxin variants have lower toxicity. Young rats are more susceptible than adults, and prior exposure appears to reduce susceptibility. There are no experimental data for subchronic exposure, reproductive, teratogenic or carcinogenic effects of saxitoxins.

Much human health data for saxitoxin poisoning is available as a result of the population and individual poisonings reported over the last 300 years. National regulatory agencies have set maximum limits for saxitoxins in shellfish for human consumption, and commercial and recreational harvesting is prohibited when shellfish samples contain saxitoxins above the regulatory limit. There does not appear to be evidence for lasting effects of poisoning by saxitoxin, unlike the effects seen following poisoning by other neurotoxic marine shellfish poisons.

Reading

A detailed current account of the health effects related to exposure to cylindrospermopsins and microcystins may be found in

Falconer IR (2005) Cyanobacterial Toxins of Drinking Water Supplies; Cylindrospermopsins and Microcystins. CRC Press, Boca Raton, FL, pp 279

A broad review of cyanobacterial toxins, including ecology and monitoring, can be found in

Chorus I , Bartram J (1999) Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. E & FN Spon, London, pp 416

A review of the entire field of marine and freshwater algal and cyanobacterial toxins can be found in

Falconer IR (Ed) (1993) Algal toxins in Seafood and Drinking Water. Academic Press, London, pp 224