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Time-dependence of lung injury in mice acutely exposed to cylindrospermopsin

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ABSTRACT

Cylindrospermopsin is a cyanobacterial toxin of increasing environmental importance, as it can lead to disease if orally or intravenously absorbed. However, its *in vivo* lung impairment has not been documented. Thus, we aimed at verifying whether cylindrospermopsin can induce lung injury and establish its putative dependence on the time elapsed since exposure.

BALB/c mice were intratracheally injected with either saline (NaCl 0.9%, 50 μL, SAL group, $n = 12$) or a sublethal dose (70 μg/kg) of semi-purified extract of cylindrospermopsin (CYN groups, $n = 52$). Lung mechanics, histological and biochemical analyses, and cylindrospermopsin presence in lungs and liver were determined in independent groups at 2, 8, 24, 48, and 96 h after cylindrospermopsin instillation.

There was a significant increase in static elastance at 24 and 48 h after exposure to cylindrospermopsin, while viscoelastic component of elastance and viscoelastic pressure rose at 48 h. Alveolar collapse augmented in CYN groups at 8 h. A significant increase in polymorphonuclear influx into lung parenchyma, as well as a higher myeloperoxidase activity started off at 24 h. Exposure to cylindrospermopsin increased lipid peroxidation and superoxide dismutase activity and reduced catalase activity in CYN groups. The toxin was detected in lungs and liver of all CYN mice.

In conclusion, cylindrospermopsin exposure impaired lung mechanics, which was preceded by lung parenchyma inflammation and oxidative stress.

1. Introduction

Cyanobacteria are a group of prokaryotic organisms primarily found in freshwater environments, especially in tropical regions, where warm water temperatures and high nutrient concentrations often allow their growth (Saker and Eaglesham, 1999). Of major concern is the production of toxins that have become recognized as potent hazards in drinking water throughout the world (Falconer and Humpage, 2006). Our previous studies with a cyanotoxin (Picanço et al., 2004; Soares et al., 2007; Carvalho et al., 2010; Casquilho et al., 2011) showed that a single intraperitoneal sub-lethal dose (40 μg/kg BW) of microcystin-LR (MCYST-LR) impairs lung mechanics and increases polymorphonuclear influx in lung parenchyma.

The toxic alkaloid cylindrospermopsin can be produced by a range of cyanobacterial species, like *Cylindrospermopsis raciborskii* (Ohtani et al., 1992),

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Aphanizomenon ovalisporum (Banker et al., 1997), *Raphidiopsis curvata* (Li et al., 2001), and *Umezakia natans* (Harada et al., 1994). In 1978, a serious poisoning of humans resulting from consumption of water contaminated with the toxic cyanobacterium *C. raciborskii* took place in Palm Island, Australia. It was known as the "Palm Island mystery disease", and well over 100 children had to be admitted to the hospital showing various symptoms of gastroenteritis (Byth, 1980; Bourke et al., 1983; Griffiths and Saker, 2003; Berry et al., 2009). Since that time a great deal of attention has been dedicated to cylindrospermopsin, although there is no data in the literature reporting the dose-dependence of human beings to that toxin. Poisoning resulted from recreation (Chorus et al., 2000; Rao et al., 2002) and possible accumulation in the food-web (Saker and Eaglesham, 1999); transmission from mice females to their fetuses (Paerl et al., 2001; Codd et al., 2005; Falconer and Humpage, 2006; Rogers et al., 2007) has also been reported.

Owing to its high solubility in water and low rate of bio- and photodegradation, significant amounts of cylindrospermopsin can be expected to occur in the water column (Wormer et al., 2008, 2010). The toxin concentrations in the European environment were found to amount up to 12.1 μg/L in Germany (Rücker et al., 2007), up to 9.4 μg/L in Spain (Quesada et al., 2006), and up to 18.4 μg/L in Italy (Bogialli et al., 2006). US EPA classified cylindrospermopsin as a compound with high priority for hazard characterization (U.S. Environmental Protection Agency, 2001).

Despite considerable research, much remains to be disclosed with respect to the toxicity of cylindrospermopsin. It is known that the toxin irreversibly inhibits protein synthesis. However, the mechanisms involved in its toxicity and metabolism are not well understood. Terao et al. (1994) reported ribosome detachment from the rough endoplasmic reticulum, and the linkage of an active metabolite of cylindrospermopsin to DNA or RNA, with consequent blockage of translation, was also suggested (Shaw et al., 2000). Cylindrospermopsin can also induce DNA fragmentation, chromosome losses, and possibly carcinogenicity (Humpage et al., 2000, 2005; Falconer and Humpage, 2001; Shen et al., 2002). Cylindrospermopsin toxicity seems to present two toxic responses (Falconer, 2008): The rapid toxicity appears to be mediated by CYP450 activation, which generates more toxic metabolites, while the longer-term toxicity is due to protein synthesis inhibition (Humpage et al., 2005).

Although lethal doses of cylindrospermopsin can damage the liver, kidney, lung, heart, stomach and the vascular system (Hawkins et al., 1985), there are no reports in the literature investigating *in vivo* pulmonary damage produced by sub-lethal doses of cylindrospermopsin. Moreover, the understanding of the effects of these doses of the toxin is relevant because human beings are often exposed to low doses of cyanotoxins. Hence, in the present study we aimed at verifying whether a single sub-lethal dose of cylindrospermopsin can induce lung injury, and establish its putative dependence on the time elapsed since exposure.

2. Materials and methods

2.1. Animals

BALB/c male mice (6–7 week of age) were purchased from CEMIB (Multidisciplinary Center for Biological Investigation, University of Campinas, Campinas, Brazil). The animals were housed in plastic cages with absorbent bedding material and maintained on a 12-h daylight cycle. Food and water were provided *ad libitum*. The experimental protocol was approved by the Ethics Committee on the Use of Animals, Health Sciences Center, Federal University of Rio de Janeiro (Protocol IBCCF 012).

2.2. Experimental procedures

Two separate experiments, with equal procedures, were necessary for this study. The first one used thirty-four mice, randomly divided into 6 groups (5–6 animals per group) for pulmonary mechanics and histological analyses. The second experiment had 30 animals sacrificed for all biochemical analyses.

We had 4 control animals at all time points in the first set of experiments. After running a one-way ANOVA followed by Bonferroni's multiple comparisons test using the mechanics data, all control groups were statistically similar. Thus, one animal was randomly picked up from each group and, thus, SAL group was formed ($n = 5$). In the second batch of animals 5 mice were used as controls. SAL animals received a single intratracheal instillation (i.t.) of 50 μL of saline solution (NaCl 0.9%). Cylindrospermopsin groups (CYN) received a single sublethal dose of semi-purified extract of cylindrospermopsin (70 μg/kg body weight, i.e., 45–55 μL, i.t.). This dose was chosen based on the cylindrospermopsin LD_{50} in mice (i.p.), namely, 200 μg/kg BW (Terao et al., 1994). All animals (25–30 g) were analyzed 2, 8, 24, 48 and 96 h after instillation. For intratracheal instillation mice were anesthetized with sevoflurane, and saline or cylindrospermopsin was gently instilled into their tracheas with the aid of an ultra-fine U-100 insulin syringe. The animals rapidly recovered after instillation.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Academy of Sciences, USA.

2.3. Cylindrospermopsin semi-purified extract production

The animals were exposed to a semi-purified extract of *C. raciborskii*. The cylindrospermopsin producer strain CYP 011K, kindly provided by Dr. Andrew Humpage and Dr. Peter Baker (Australian Water Quality Centre, Adelaide, Australia) was cultured in ASM-1 medium, the lyophilized biomass was extracted in ultrapure water, centrifuged and passed through a C18 cartridge to remove part of the matrix interference. The process ensured the removal of any cyanobacterial LPS in the extract. The extraction step and HPLC analysis of toxin content were done according to Welker et al. (2002).