Harmful Algae 37 (2014) 68–74

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Oxidative stress in rats induced by consumption of saxitoxin contaminated drink water

Patrícia Baptista Ramos [a], Felipe Diehl [a], Juliane Marques dos Santos [a], José Maria Monserrat [b,*], João Sarkis Yunes [a]

[a] Programa de Pós-graduação em Oceanografia Física, Química e Geológica, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, CEP 96203-900, Cx. P. 474, Rio Grande, RS, Brazil
[b] Programa de Pós-graduação em Fisiologia Animal Comparada, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande – FURG, CEP 96201-900, Cx. P 474, Rio Grande, RS, Brazil

ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form 8 January 2014
Accepted 7 April 2014

Keywords:
Brain
Cylindrospermopsis raciborskii
Liver
Oxidative stress
Rats
Saxitoxins

ABSTRACT

Saxitoxins (STXs) are neurotoxins produced by cyanobacteria such as *Cylindrospermopsis raciborskii*. During bloom events, the production of these compounds causes contamination on public water supply sources. STXs block voltage gated sodium channels and can lead to severe poisoning and death of organisms at different trophic levels. Other toxicity mechanism of STX is the generation of reactive oxygen species (ROS). The aim of this study was to investigate the effect of consumption of water contaminated with a *C. raciborskii* strain (producing variants of Neo-STX and STX) by rats during 30 days through the analysis of oxidative stress biochemical parameters. Total antioxidant capacity (ACAP) and oxidative stress parameters were analyzed at pre-frontal cortex, hippocampus and liver of adult Wistar rats (2–3 months old). Treated animals ingested concentrations of 3 and 9 μg/L of STX equivalents and were compared with a control group (culture medium ASM-1). At the concentration of 3 μg/L, a decrease in ROS production associated with lower ACAP at hippocampus was observed. Furthermore, a decrease of glutamate cysteine ligase (GCL) activity in the cortex and an increase of brain and liver glutathione concentration were also observed. At the highest concentration (9 μg/L), there was an ACAP increase in the hippocampus as well as in the activity GCL and glutathione-S-transferase in the cortex and hippocampus. At both concentrations, lipid peroxidation was registered in the liver. Therefore, chronic ingestion of STXs can alter the antioxidant defenses and induce oxidative stress in brain and liver. The present results point to the values adopted as threshold limit for STXs in potable waters (3 μg/L) shows already significant chronic effects that alter antioxidant defenses and induce oxidative stress at least in two of the organs studied.

1. Introduction

Saxitoxins (STX) comprise a group of neurotoxins of different isoforms with a tetrahydropurine structure known as paralytic shellfish toxins (PSTs) (Clemente et al., 2010). They are produced in marine environments by dinoflagellates also responsible for the red tides (Oshima et al., 1993) and by freshwater cyanobacteria (Carmichael et al., 1997; Molica et al., 2005). More than 24 STX analogs have been described, according Oshima et al. (1993). These molecules present a varied toxicity due to side chain residues such as hydroxyl, sulfate and carbamoyl, which allow the division of STXs into three classes (Oshima et al., 1993; Araoz et al., 2010; Silva et al., 2011). The most potent toxins are the saxitoxins (STX) (relative toxicity: 1.0), neosaxitoxin (NeoSTX) (relative toxicity: 0.92) and gonyautoxins (GTX1-4) (relative toxicity: 0.36–0.99) (Asp et al., 2004). Blooms of *Cylindrospermopsis raciborskii* producing saxitoxins (STX) have been frequent in reservoirs used for public water supply in Brazil, and constitute a serious public health risk (Yunes et al., 2003; Molica et al., 2005). Lagos et al. (1999) confirmed the presence of PST analogs in three different strains of *C. raciborskii* (T1, T2, T3) isolated from a dam in the state of São Paulo, with the variants NeoSTX and STX, considered the most toxic, being identified in strain T3. As a result of these events, the Brazilian Ministry of Health (Regulation number 2914/2011)

* Corresponding author at: Instituto de Ciências Biológicas (ICB), Universidade Federal do Rio Grande – FURG, CEP 96201-900, Cx. P 474, Rio Grande, RS, Brazil. Fax: +55 5332936856.
E-mail address: josemmonserrat@pq.cnpq.br (J.M. Monserrat).

http://dx.doi.org/10.1016/j.hal.2014.04.002
1568-9883/

made saxitoxins analysis in drinking water mandatory, with the maximum acceptable limit of 3.0 μg/L (Fitzgerald et al., 1999; Brazilian Ministry of Health, 2011).

Freshwater mussels and marine clams tend to accumulate STX in high concentrations by filter-feeding dinoflagellate and toxic cyanobacteria (Evans, 1971; Nogueira et al., 2004; Araoz et al., 2010; Zhang et al., 2011). Galvao et al. (2009) observed STX accumulation in tilapia fish *Oreochromis niloticus*, used for human consumption. Symptoms of PST poisoning in humans by PSTs include paralysis of mouth and extremities, dizziness, muscle weakness, nausea, vomiting, tachycardia, followed by death in severe cases due to cardio-respiratory arrest (Negri et al., 1995; Araoz et al., 2010; Etheridge, 2010; Zhang et al., 2011). In addition to these systemic effects, the major toxic effects of STX are found in the brain (Cervantes Cianca et al., 2007), since they are capable of crossing the blood–brain barrier (BBB) (Andrinolo et al., 1999). Accumulation of STX in the central nervous system (Andrinolo et al., 1999; Cervantes Cianca et al., 2007) has already been reported, even though the BBB transport mechanism is still unknown. STX have affinity for the voltage-gated sodium channel present on excitable membranes of neurons and myocytes, thereby affecting their ion permeability and resistance inducing neuromuscular paralysis and death from respiratory arrest (Araoz et al., 2010; Etheridge, 2010).

The generation of reactive oxygen species (ROS) is an inherent condition to aerobic life (Jones, 2006; Pamplona and Costantini, 2011). ROS have dualistic role as they are involved in protective mechanisms of the cell (Cooke et al., 2003) and act as messengers in cellular signaling pathways (Pamplona and Costantini, 2011), but at high concentrations they can induce deleterious effects on macromolecules including proteins, lipids and DNA (Jones, 2006; Rosenfeldt et al., 2013). It is well known that many xenobiotics such as cyanotoxins can not only cause an overproduction of ROS but also alter the levels of antioxidants, which may lead to a condition of oxidative stress (Ding and Ong, 2003; Pinho et al., 2005; Leão et al., 2008; Amado et al., 2009). Although scarce, some studies demonstrate the STX toxicokinetics induce oxidative stress. According to Estrada et al. (2007), the dinoflagellate *Gymnodinium catenatum*, a saxitoxins producer, caused alteration of antioxidant enzymes and induction of oxidative stress in different tissues of the shellfish *Nodipecten subnodosus*. Gubbins et al. (2000) and Nogueira et al. (2004) showed that STX increase glutathione-S-transferase (GST) activity in fish and cladocerans, suggesting that GST plays an important role in the detoxification process of such toxins. Since GST is related to antioxidants responses, these results can also be indicative of a possible relation between saxitoxin poisoning and oxidative stress (Clemente et al., 2010; Choi et al., 2006). Silva et al. (2011) observed that saxitoxins can be bioaccumulated along the food chain, decreasing in the activity of superoxide dismutase (SOD), GST and glutathione peroxidase (GPx) and causing oxidative stress in the brain of the fish *Hoplias malabaricus*, leading to damage of lipids, proteins and DNA. Melegari et al. (2012) showed that the saxitoxins induce oxidative stress through lipoperoxidation and by decreasing the activity of some antioxidant enzymes in the Neuro-2A (N2A) animal cell culture. In this same study, similar effects were observed in the activity of CAT and SOD in the algae *Chlamydomonas reinhardtii*. Hong et al. (2003) observed altered responses of some antioxidant enzymes such as SOD and GPx in mice liver after oral exposure to sub-lethal doses of STX.

However, there are few studies in the literature showing oxidative stress in mammals after sub-lethal and repeated oral exposure to STX. Thus the objective of this study was to investigate the effect of consumption of water contaminated with a strain of *Cylindrospermopsis. raciborskii*, a producer of saxitoxins, in concentrations of 3 and 9 μg/L for 30 days on biochemical parameters associated with oxidative stress of the brain and liver of rats.

2. Materials and methods

2.1. Biological model

Female Wistar rats (2–3 months-old and 210–300 g weight; $N = 120$) were housed in plastic cages (39 cm × 32 cm × 17 cm), five per cage, under a 12 h light/dark cycle and at a constant temperature of 24 ± 1 °C with water and food supplied *ad libitum*. Only female Wistar rats were employed in the experiments. Experiments with rats were performed in strict accordance to the Brazilian law, the Brazilian College of Animal Experimentation (COBEA) and approved by the Universidade Federal do Rio Grande – FURG Ethics Committee (process number P025/2011 CEUA/FURG). Number of replicates employed in the biochemical measurements ranged from 5 to 15.

2.2. Toxins source

Cylindrospermopsis raciborskii cells from the Culture Collection of the Cyanobacteria Research Unit (UPC) were grown in ASM-1 (Gorham et al., 1964) at 25 ± 1 °C under continuous cool-white fluorescent light (intensity: 127 μE m^{-2} s^{-1}). Under such conditions the strain produces variants of neoSTX and STX detected by HPLC–FLD, which were analyzed and compared with the commercial standard of STX, neoSTX and dc-STX (NRC, Canada). For extraction of toxins, cells of *C. raciborskii* were sonicated (three cycles of 1 min each) in order to break the cellular walls and release the toxins. The aqueous extract was then centrifuged at 10,000 × *g* at 4 °C during 10 min and the supernatant retired for toxin quantification by HPLC.

2.3. Experimental design

It was offered to the experimental model water with cyanobacterial cultures during 30 days (thus representing a chronic oral systemic administration) using drinking water bottles (800 mL). Five animals from each cage drank an average of 400 mL/day and no significant differences in consumption were observed between the experimental groups. Every week during the 30-day treatment period, the STX concentration (3 or 9 μg/L) in the drinking water bottles was verified by HPLC/FLD. Each rat drank 0.24 or 0.72 μg of STX/day of 3 or 9 μg/L treatment oral tests. STX concentrations in the bottles remained constant for at least a week. Only and when it was necessary, concentration correction to 3 or 9 μg/L was made with dilutions containing growth medium.

In all the experiments conducted, there were always three distinct treatments: (1) control: culture medium ASM-1 strain of *Cylindrospermopsis raciborskii* ($n = 5–10$), (2) water contaminated with 3 μg/L of STX ($n = 5–10$), which is the maximum concentration allowed by the Regulation 2914/2011 of the Ministry of Health for drinking water, and (3) water contaminated with 9 μg/L of STX ($n = 5–10$), a concentration three times higher than that allowed by Brazilian legislation. At the end of 30 days, the rats were killed and organ dissected for biochemical measurements.

2.4. Measurement of biochemical variables

2.4.1. Tissue samples preparation

After the period of exposure to STXs, rats were anesthetized with ketamine and xylazine intraperitoneally at doses of 75 and 10 mg/kg, respectively. Subsequently, the animals were decapitated and had their brain (hippocampus and prefrontal cortex) and liver quickly removed. Tissue samples used in measurements involving enzymatic activities were prepared by homogenization (1:5 w/v) in Tris–HCl buffer (100 mM, pH 7.75), EDTA (2 mM) and $MgCl_2 \cdot 6H_2O$ (5 mM) (Gallagher et al., 1992). Homogenates were centrifuged at 10,000 × *g* for 20 min at 4 °C and the resulting