Harmful Algae 64 (2017) 20–29

Contents lists available at ScienceDirect

Harmful Algae

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

Chronic low-level exposure to the common seafood toxin domoic acid causes cognitive deficits in mice

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ARTICLE INFO

Article history:
Received 9 January 2017
Received in revised form 15 March 2017
Accepted 17 March 2017
Available online xxx

ABSTRACT

The consumption of one meal of seafood containing domoic acid (DA) at levels high enough to induce seizures can cause gross histopathological lesions in hippocampal regions of the brain and permanent memory loss in humans and marine mammals. Seafood regulatory limits have been set at 20 mg DA/kg shellfish to protect human consumers from symptomatic acute exposure, but the effects of repetitive low-level asymptomatic exposure remain a critical knowledge gap. Recreational and Tribal-subsistence shellfish harvesters are known to regularly consume low levels of DA. The aim of this study was to determine if chronic low-level DA exposure, at doses below those that cause overt signs of neurotoxicity, has quantifiable impacts on cognitive function. To this end, female C57BL/6NJ mice were exposed to asymptomatic doses of DA (≈0.75 mg/kg) or vehicle once a week for several months. Spatial learning and memory were tested in a radial water maze paradigm at one, six and 25 weeks of exposure, after a nine-week recovery period following cessation of exposure, and at three old age time points (18, 24 and 28 months old). Mice from select time points were also tested for activity levels in a novel cage environment using a photobeam activity system. Chronic low-level DA exposure caused significant spatial learning impairment and hyperactivity after 25 weeks of exposure in the absence of visible histopathological lesions in hippocampal regions of the brain. These cognitive effects were reversible after a nine-week recovery period with no toxin exposure and recovery was sustained into old age. These findings identify a new potential health risk of chronic low-level exposure in a mammalian model. Unlike the permanent cognitive impacts of acute exposure, the chronic low-level effects observed in this study were reversible suggesting that these deficits could potentially be managed through cessation of exposure if they also occur in human seafood consumers.

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1. Introduction

Domoic acid (DA) is a neurotoxic amino acid that is naturally produced by some species of diatoms of the genus *Pseudo-nitzschia* found in phytoplankton communities in oceans throughout the world (Bates, 2000; Lundholm and Moestrup, 2000; Marchetti et al., 2008; Martin et al., 1990). During harmful algal blooms (HABs) of toxigenic *Pseudo-nitzschia*, DA can accumulate in filter-feeding fish and shellfish making them unsafe for consumption by marine mammals and humans (Scholin et al., 2000; Todd, 1993). Current evidence suggests that HABs in general are increasing in magnitude and frequency globally as oceans continue to warm (Moore et al., 2008; Van Dolah, 2000). In 2015, an unprecedented coastwide toxic *Pseudo-nitzschia* bloom was linked to a warm water anomaly that spanned the west coast of North America resulting in large scale closures of razor clam (*Siliqua patula*), rock crab (*Cancer anthonyi*, *C. antennarius*, and *C. productus*) and Dungeness crab (*C. magister*) fisheries in multiple states (McCabe et al., 2016). Record-breaking levels of DA were detected in coastal food webs and seafood resources resulting in devastating economic losses and ecological damage (McCabe et al., 2016).

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http://dx.doi.org/10.1016/j.hal.2017.03.003
1568-9883/Published by Elsevier B.V.

Domoic acid was first recognized as a seafood toxin in 1987 when over 100 people became ill after consuming DA-contaminated mussels (*Mytilus edulis*) harvested near Prince Edward Island in Canada (Perl et al., 1990). The condition was termed amnesic shellfish poisoning (ASP) and symptoms included gastrointestinal distress, confusion, disorientation, seizures, permanent short-term memory loss, and in the most severe cases death (Perl et al., 1990). In the aftermath of this event, a seafood safety regulatory limit was set at 20 mg DA/kg shellfish (Marien, 1996). The regulatory limit was based on the lowest level of DA reported to cause observable toxic effects from mussel consumption in the Canadian event (1 mg DA/kg body weight) and oral dose studies with primates where effects were not observed at 0.5 and 0.75 mg/kg body weight doses, but clinical effects were observed at 1 mg/kg body weight doses (Iverson et al., 1990; Marien, 1996; Wekell et al., 2004). The acute reference dose (ARfD) for acute DA exposure was determined as 0.075 mg/kg by taking 0.75 and applying a safety factor of 10. This limit was set to protect human health from acute toxicity in the context of one meal of 270 g of clams in a 70 kg adult and includes a 10-fold safety factor to protect sensitive individuals such as young, old, and health compromised (Marien, 1996). It does not take into account consumption over multiple days, larger meal sizes, and/or potential effects of chronic long-term low-level (below the regulatory limit) toxin exposure. Although the risks of acute exposure to symptomatic doses of DA have been minimized by testing shellfish and regulating harvests based on toxin loads ($\geq$ 20 mg DA/kg shellfish = harvest closure), there are no regulations in place for protection from long-term low-level repetitive DA exposure ($<$ 20 mg DA/kg shellfish = harvest open). The effects of chronic low-level asymptomatic exposure have not been well studied and are of particular concern for coastal communities including recreational and Tribal harvesters who regularly consume shellfish such as razor clams that are known to retain low levels of DA in edible tissues for more than a year after HAB events (Lefebvre and Robertson, 2010; Wekell et al., 1994). In a study with long-term whole animal exposures to low asymptomatic doses of DA, zebrafish (*Danio rerio*) had altered gene transcriptomes and impaired mitochondrial function in whole brains after nine months of weekly exposures (Hiolski et al., 2014). Additionally, chronic low-level exposure did not confer resistance to DA, but instead increased toxin sensitivity making the neurologic effects of subsequent exposures more pronounced in chronically exposed animals (Hiolski et al., 2014).

In addition to human health risks, DA poisoning is a significant health risk for marine mammals that is likely increasing as HABs increase. The first DA poisoning event documented in marine mammals occurred in 1998 in Monterey Bay, CA when hundreds of California sea lions (*Zalophus californianus*) stranded on beaches exhibiting signs of neurotoxicity in the form of seizures, tremors and ataxia (Scholin et al., 2000). Over the last 18 years following the first documented DA poisoning event in marine mammals, dozens to hundreds of sea lions continue to be sickened each year with exceptionally high numbers in the most recent years (Bejarano et al., 2008; McCabe et al., 2016). From 2010–2013, The Marine Mammal Center in Sausalito, CA diagnosed an average of 64 $\pm$ 21 sea lions per year with DA poisoning, while numbers rose to over 200 in 2014 and 2015 (McCabe et al., 2016). Sea lions have served as a valuable sentinel for human health and a mammalian model for the elucidation of mechanisms of DA toxicity with natural environmental exposure. Acute toxicity as described above results from DA exposure high enough to elicit outward signs of neuroexcitotoxicity such as seizures within hours after exposure. A persistent toxicity syndrome has also been defined for neurologic effects that continue long after an initial acute exposure and is characterized by episodic seizures and permanent spatial memory loss (Cook et al., 2015; Goldstein et al., 2008; Muha and Ramsdell, 2011). As with humans, the effects of low-level repetitive exposure (levels below those that elicit visible neurotoxic symptoms) have not been studied in marine mammals and are likely to impact chronically exposed animals.

The objectives of this study were to determine if chronic asymptomatic DA exposure at doses below those that cause the overt signs of neurotoxicity described above leads to underlying health impacts in the mammalian system. To this end, female C57BL/6NJ mice were chronically exposed to low asymptomatic doses of DA via intraperitoneal injection (IP) once a week beginning at 3 months of age and examined at multiple time points for spatial memory and learning as well as general activity levels and gross morphology in hippocampal regions of the brain. Results of this study are applicable to both human and marine mammal health.

2. Methods

2.1. Test animals

Eight-week-old male and female C57BL/6NJ mice were obtained from The Jackson Laboratory and acclimated for four weeks. All mice were housed at the animal research facility at the University of Washington and provided free access to a standard rodent diet (PicoLab® Rodent Diet 20, Lab Diet, USA) and water *ad libitum* in a controlled environment with a 12-h light/dark cycle. Dose response assays were performed with male and female mice to determine if there were sex differences in toxin susceptibility using scratching as a toxicological endpoint (Tasker et al., 1991). Five IP doses were used to calculate 50% effective concentrations (EC50s) for male and female mice for comparison of DA sensitivity (n = 4 mice per sex per dose). Sex differences were not observed and female mice were chosen for subsequent long-term chronic exposure experiments because females were easier to handle during injections. Additional eight-week-old female C57BL/6NJ mice were purchased from The Jackson Laboratory and allowed to acclimate for four weeks before long-term asymptomatic exposure studies began. Animal handling and experimental procedures were performed in accordance with protocols approved by the Animal Care and Use Committee at the University of Washington.

2.2. Quantification of asymptomatic exposure doses

The target asymptomatic exposure dose was chosen based on EC50 values and results from a pilot study where mice were injected weekly for six weeks with doses approximately 40% of the EC50 to identify a dose that would not elicit overt signs of neurotoxicity after repetitive exposure. All DA solutions were prepared from powdered DA (purchased from Tocris and from Sigma Aldrich) dissolved in nanopure water and quantified via high performance liquid Chromatography (HPLC) (Quilliam et al., 1989). Stock solutions were then diluted to target concentrations in phosphate buffered saline (PBS), analyzed by HPLC to confirm target doses and administered via IP injections of 200 μl in mice. Mice were weighed at all injections and doses were quantified for all animals at all time points.

2.3. Chronic exposure experiments

Female C57BL/6NJ mice were IP-injected once a week with either PBS (control) or approximately 0.75 to 0.82 mg DA/kg body weight from three months of age to nine months of age (25 weeks of exposure). One cohort of mice was sampled at one week and six weeks (n = 10 exposed, n = 10 control) of exposure and a second separate cohort of mice was sampled at 25 weeks (n = 20 exposed, n = 20 control) of exposure and all were tested for spatial learning