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Neurobehavioral assessment of mice following repeated oral exposures to domoic acid during prenatal development

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ABSTRACT

Domoic acid (DA) is an algal toxin which has been associated with significant neurotoxicity in humans, non-human primates, rodents, and marine mammals. Developmental exposure to DA is believed to result in neurotoxicity that may persist into adulthood. DA is produced by harmful algal blooms of *Pseudo-nitzschia*, raising concerns about the consumption of contaminated seafood. We evaluated oral exposures to DA during pregnancy in mice. Doses of 0 (vehicle), 1 or 3 mg/kg/d of DA were administered by gavage to C57BL/6J mice on gestational days 10 to 17. The offspring were tested for persistent neurobehavioral consequences during early development, adolescence and adulthood. Neurobehavioral tests revealed both dose- and gender-related differences in several neurobehavioral measures, including motor coordination in the rotarod test, behavior in the elevated plus maze, circadian patterns of activity in Phenotyper cages, gait as assessed in the Catwalk, and exploratory activity in the Morris water maze. This study demonstrated significant gender-specific and persistent neurobehavioral effects of repeated prenatal oral exposures to DA at low-dose levels that did not induce toxicity in dams.

1. Introduction

Domoic acid (DA) is an algal toxin which causes significant neurotoxicity in multiple species, such as humans, non-human primates, rodents, and marine mammals (Iverson et al., 1989, Tryphonas et al., 1990a, Tryphonas et al., 1990b, Tasker et al., 1991). Since cultured mussels contaminated with DA from Prince Edward Island, Canada, induced severe toxicity in humans in 1987, studies have been conducted focused on the various aspects of its toxicity, particularly to the central nervous system. DA is a structural analog of kainic acid (KA), an excitatory amino acid that exerts its toxicity by activating the AMPA/KA subtype of glutamate receptors (Hampson and Manalo, 1998). Currently, guidelines to protect human public health have been implemented to limit DA contamination in shellfish. However, the guidelines, such as the acute reference dose (ARfD) and tolerable daily intake (TDI), are based on studies in adults (Marien, 1996). Concerns remain whether fetuses, infants and/or children show higher susceptibility than adults. DA is known to cross the placental barrier and has been detected in amniotic fluid, fetal blood, and fetal brain tissue in rats (Maucher and Ramsdell, 2007, Maucher Fuquay et al., 2012). Although a number of in vitro and in vivo studies have investigated the potential developmental neurotoxicity of DA, investigations following prenatal exposures are still limited.

During development, mRNA expression of individual KA receptor subunits (such as AMPA/KA) can be detected on gestational day (GD) 11.5 to 13.5 in mouse brain (Data in Allen institute for Brain Science Inc., http://www.alleninstitute.org). The mRNA for AMPA receptor subunits, such as GluR-A, GluR-B, and GluR-C, are detectable from GD 13 in rat brain (Lilliu et al., 2001). Exposures to DA around GD13 have been shown to induce persistent effects on neuronal development. For instance, intrauterine exposure of mice to DA on GD 13, at a dose level that did not result in overt behavioral abnormalities in the dams,

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resulted in progressive hippocampal injury to the pups on post-natal day (PND) 14 or later, and electroencephalogram (EEG) alterations on PND 30 (Dakshinamurti et al., 1993). Neurochemical analyses on PND 30 also revealed an increase in KA receptors and glutamate levels and a decrease in GABA levels in the brain. Additional results indicated enhanced Ca^{2+} influx into cortical and hippocampal slices isolated from the offspring of exposed mice, suggesting that intrauterine exposure to DA on GD13 can induce hippocampal excitotoxicity by increasing neuronal calcium influx through KA receptors in the pups (Dakshinamurti et al., 1993). Another study in rats demonstrated that prenatal exposure to DA on GD 13 caused impairments in the locomotor and cognitive domains at 4 to 13 weeks of age (Levin et al., 2005). Tanemura et al. (2009) reported that mice given DA on GD 11.5 or 14.5 exhibited significant deficits in anxiety-related behaviors, severe impairment in learning and memory, and/or morphological changes such as myelination failure and overgrowth of neuronal dendrites in the cerebral cortex and hippocampus at 11 weeks of age. Finally, Zuloaga et al. (2016) reported decreased pre-pulse inhibition in mice prenatally exposed DA on GD 14, 15 or 16 (Zuloaga et al., 2016). These findings suggest that prenatal exposure to DA at levels that do not cause symptoms in exposed dams during critical stages of fetal neurogenesis can result in brain alterations and behavioral disturbances that persist into adulthood. The brain alterations associated with prenatal exposure to DA can sometimes be more pervasive than those associated with postnatal exposure.

It should be noted that in all of the previous pre- or postnatal exposure studies, DA was administered by the intravenous, intraperitoneal, or subcutaneous routes. When considering risk assessment for humans, studies involving oral administration of DA are preferred because oral administration is the primary route of exposure to DA in humans. Also, no published prenatal exposure studies have investigated the effects of DA after repeated exposures. Rather than single administration of a high dose of DA, repeated administration of DA at low levels of exposure where acute syndrome is not produced would be most relevant for evaluating the effects of DA on children's health.

In the present study, the effects of prenatal exposure of mice to low levels of DA administered from GD 10 to 17 were investigated using multiple measures of behavior. Pregnant female mice were exposed orally to DA and the offspring were tested for persistent neurobehavioral consequences during neonatal development, adolescence, and adulthood.

2. Materials and methods

2.1. Animals and treatments

Timed-pregnant C57BL/6J mice (Charles River, CA, USA) were housed in a centralized, AAALAC-accredited, Specific Pathogen Free facility at the University of Washington. Mice that were positive for vaginal copulatory plugs [designated as gestational day (GD) 0] were shipped from Charles River on GD 7 and arrived at the University of Washington centralized vivarium on GD 8. After 48-hour acclimation, the pregnant females (GD 10) were randomly divided into 0 (control), 1 or 3 mg/kg/d dose groups for exposures to DA (D6152, Sigma-Aldrich, St. Louis, MO, USA). Purity of DA was 90% or higher with HPLC grade and all DA had the same lot number. We used Triple Quad LC/MS 6460 AAA (Aligent Technologies, Inc., Palo Alto, CA) to measure DA. Animals were housed four or five per cage until visibly pregnant, at which time they were housed individually. Mice were maintained in a room with a controlled temperature of 23 ± 2 °C, a relative humidity of 50 ± 10%, and a 12-h light-dark cycle (light on at 07:00 AM) with unlimited access to breeder chow (LabDiet; St. Louis, MO, USA) and water. All mice were housed with plastic igloos for environmental enrichment. A pilot experiment was conducted with various doses of DA (1 to 15 mg/kg/d body weight) administered to mice orally for 8 days to assess the level of toxicity in non-pregnant female mice (unpublished data). At the highest dose of DA tested (15 mg/kg/d), 2 out of 4 females developed severe paralysis of the legs after the 6th administration, and another female was found dead on the morning of the 8th day. At 5 mg/kg/d, 1 out of 4 females showed abnormal gait after the 8th administration. Therefore, we decided to use non-symptomatic dose levels of DA (1 and 3 mg/kg/d) to assess subchronic toxicity of DA to pregnant mice in the main study. OECD guidelines for the testing of chemicals (OECD TG) 452 (2009) indicate that variability in test volume should be minimized by adjusting the concentration of the chemical to ensure a constant volume at all dose levels. For this study, DA was dissolved in sterile distilled water at different concentrations such that a volume of 150 µl/animal of the solution was administered to produce a dose of either 1 or 3 mg/kg. The DA solutions were prepared daily based on the body weights in the morning, and administered via oral gavage to the mice daily from GD 10 to 17, using a 20G × 3.5-cm stainless steel curved animal feeding needle. Control animals received 150 µl/animal of vehicle (sterile distilled water) in the same manner.

The dams were allowed to deliver spontaneously and rear their offspring until weaning on postnatal day (PND) 21. The day of delivery was designated as PND 0. To avoid unnecessary stress to dams, litters were left undisturbed until PND 4. On PND 4, gender and weight of every pup was recorded and litters were culled randomly to 2 males and 2 females for the behavior testing. Culled pups were saved for biochemical and molecular analyses. After weaning, the pups were group-housed 2 to 4 per cage for the duration of behavioral testing. Same sex littermates were housed together. There were 18 litters used in two cohorts, with 6 litters per dose group (or 12 pups per sex/group).

All experiments were approved by the University of Washington Institutional Animal Care and Use Committee (UW IACUA 2017-10) and carried out in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health. We have met ARRIVE guidelines (Kilkenny et al., 2010).

2.2. Body weight and physical landmarks

The dams were weighed daily from their arrival on GD 8 until the last dosing day on GD 17. Body weights of pups were recorded every other day from PND 4 to 18 and on the weaning day (PND 21), then once a week thereafter until PND 89. The following developmental landmarks were observed daily, starting on PND 7: hair emergence, incisor eruption, opening of both eyes, descent of both testes, and vaginal opening.

2.3. Behavioral tests

The behavioral tests and ages at testing are listed in Table 1. The behavioral tests were performed during the light period by investigators who were blind to the dosing groups. All mice in the study underwent each of the behavior tests described below. Testing was designed to allow analysis of multiple behavioral domains, with use of automated testing systems whenever possible. Potentially confounding environmental variables were controlled whenever possible to minimize variability. These controlled variables included temperature, humidity, diet, lighting, extraneous noise, odors, and housing type and method. All animals were handled repeatedly (at least once daily) throughout the postnatal developmental period.

2.4. Pre-weaning tests

Pups were tested for reflex development and neuromotor ability by assessing reflex righting, cliff avoidance and negative geotaxis (Adams, 1986; Moser, 2001). Beginning on PND 5, pups were tested daily for reflex righting by measuring the time to right onto all four paws after being placed in a supine position. Reflex righting was tested once daily