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Fluoxetine treatment ameliorates depression induced by perinatal arsenic exposure via a neurogenic mechanism

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ABSTRACT

Several epidemiological studies have reported an association between arsenic exposure and increased rates of psychiatric disorders, including depression, in exposed populations. We have previously demonstrated that developmental exposure to low amounts of arsenic induces depression in adulthood along with several morphological and molecular aberrations, particularly associated with the hippocampus and the hypothalamic–pituitary–adrenal (HPA) axis. The extent and potential reversibility of this toxin-induced damage has not been characterized to date. In this study, we assessed the effects of fluoxetine, a selective serotonin reuptake inhibitor antidepressant, on adult animals exposed to arsenic during development. Perinatal arsenic exposure (PAE) induced depressive-like symptoms in a mild learned helplessness task and in the forced swim task after acute exposure to a predator odor (2,4,5-trimethylthiazoline, TMT). Chronic fluoxetine treatment prevented these behaviors in both tasks in arsenic-exposed animals and ameliorated arsenic-induced blunted stress responses, as measured by corticosterone (CORT) levels before and after TMT exposure. Morphologically, chronic fluoxetine treatment reversed deficits in adult hippocampal neurogenesis (AHN) after PAE, specifically differentiation and survival of neural progenitor cells. Protein expression of BDNF, CREB, the glucocorticoid receptor (GR), and HDAC2 was significantly increased in the dentate gyrus of arsenic animals after fluoxetine treatment. This study demonstrates that damage induced by perinatal arsenic exposure is reversible with chronic fluoxetine treatment resulting in restored resiliency to depression via a neurogenic mechanism.

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1. Introduction

In a recent report by the ATSDR (Agency for Toxic Substances and Disease Registry), arsenic was singled out as a considerable threat to human health compared to other toxicants, outranking even mercury and lead. While the World Health Organization standard remains at 10 parts per billion (ppb), it is estimated that over 100 million people worldwide are exposed to arsenic in amounts that well exceed this standard (in the ppm range) (Tyler and Allan, 2014). Research over the past few decades has identified pathologies resulting from chronic arsenic exposure, demonstrating that this metalloid disrupts many organ systems in the body, including the brain (Brinkel et al., 2009; Farzan et al., 2013). Epidemiological studies report that cumulative long-term exposure to low doses of arsenic correlates with learning and memory deficits in children and increased rates of depression in adults (Zierold et al., 2004; Rosado et al., 2007; O'Bryant et al., 2011; Roy et al., 2011). Additionally, in almost every country assessed, individuals residing in arsenic-affected areas report a poorer quality of life, poorer mental health, and are more likely to have a mood disorder, such as anxiety or depression, than individuals without significant arsenic exposure (Fujino et al., 2004; Sen and Sarathi Biswas, 2012; Syed et al., 2012).

Research investigating the role of environmental factors, including toxin exposure, in the etiology of depression has gained momentum, as heritability studies report high discordance of the disorder and individual variability in gene expression (Byrne et al., 2013). The early environment during development has been demonstrated to exert profound effects on the modulation of behavior and susceptibility to psychiatric disorders in adulthood (Hochberg et al., 2011). In several rodent studies, pre- and postnatal toxin exposure results in the behavioral endophenotypes of anxiety and depression (Johansson et al., 2007; Onishchenko et al., 2008; Haider et al., 2013). These toxin-induced behaviors are

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often accompanied by concurrent abnormalities in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis (Hellemans et al., 2008, 2010; Bolton et al., 2013). Individuals with robust homeostatic responses to stress are often considered resistant to depression (Krishnan and Nestler, 2008), thus dysregulation of the HPA axis after toxin exposure may be a mechanism by which toxins, like arsenic, increase susceptibility to psychiatric disorders. Indeed, we have previously demonstrated that perinatal (pre- and postnatal developmental) arsenic exposure (PAE) alters glucocorticoid signaling within the HPA axis, blunts stress responses, increases stress reactivity (Goggin et al., 2012), and results in depressive-like behavior in adulthood (Martinez et al., 2008; Martinez-Finley et al., 2009). In addition to these aberrations, we have also observed altered hippocampal morphology and deficits in adult hippocampal neurogenesis after PAE (Tyler and Allan, 2013). Yet, the pathophysiology of arsenic-induced depression and the response to antidepressants after arsenic exposure has not been fully explored.

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) antidepressant, is the most commonly prescribed treatment for depression. In addition to attenuation of stress-induced depressive-like behaviors, fluoxetine contemporaneously increases adult hippocampal neurogenesis (AHN) in rodents (Wang et al., 2008; Malberg and Duman, 2003; David et al., 2009; Mahar et al., 2014). Impaired AHN has been intimately linked with the onset of depression: recent clinical research reported that depressed patients treated with fluoxetine do not exhibit hippocampal atrophy or reduced cell numbers as those patients without treatment, further supporting the "neurogenic hypothesis of depression" (Boldrini et al., 2013). Fluoxetine treatment has also been shown to normalize the corticosterone (CORT) response associated with aberrant HPA signaling after prenatal stress (Ishiwata et al., 2005); restoration of HPA axis function is associated with robust increases in neurogenesis in cell culture as well (Anacker et al., 2013). However, the molecular and behavioral responses to fluoxetine in a rodent model with arsenic-induced hippocampal morphological and behavioral deficits have not been determined to date.

To investigate the reversibility of arsenic-induced damage, we evaluated the effect of chronic fluoxetine treatment on behavioral, morphological, and molecular impairments resulting from PAE. To this end, we measured survival and differentiation of neural progenitor cells (NPCs) along with expression of several proteins involved in neurogenesis specifically in the dentate gyrus (DG) to determine fluoxetine's mechanism of action in arsenic-exposed animals. We further characterized the vulnerability to stress-induced depression generated by arsenic exposure by evaluating behavior and corticosterone (CORT) before and after brief exposure to a predator odor. Chronic fluoxetine treatment reversed deficits in the learned helplessness task; attenuated blunted stress responses and immobility after predator exposure; increased NPC survival and differentiation; and increased expression of brain-derived neurotrophic factor (BDNF), the glucocorticoid receptor (GR), histone deacetylase 2 (HDAC2), and cAMP response element binding protein (CREB) in the DG of arsenic-exposed animals but not in controls. This study suggests that impairments induced by developmental exposure to an environmental toxin like arsenic may be altered with pharmacological intervention in adulthood, leading to normalized hippocampal morphology and behavior.

2. Materials and methods

2.1. Perinatal arsenic exposure paradigm

All procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of New Mexico. Animals were maintained in a 22 °C vivarium on a 12-h reverse light/dark cycle with lights off at 08:00 and *ad libitum* access to water and food. Exposure to arsenic during all three trimesters of brain development was performed as previously described (Tyler and Allan, 2013). Briefly, singly housed female C57BL/6J mice aged 55 days (Jackson Laboratories, Bar Harbor, ME) were acclimated to drinking 50 ppb arsenic-laced water (sodium arsenate, Sigma–Aldrich, St. Louis, MO) for seven to ten days prior to mating. Dams were provided either 50 ppb arsenic water or tap water during breeding and pregnancy until offspring were weaned (Fig. 1A). Offspring were housed two per cage with tap water for the remainder of the study. The number of dams used in this study is reflected in the number of litters provided for each experimental analysis (i.e. $n = 8–10$ indicates that only one animal was used from each of 8 to 10 litters from 8 to 10 different dams). Adult male offspring (postnatal day 70) from dams drinking arsenic water were euthanized by rapid decapitation for tissue dissection or by overdose with sodium pentobarbital followed by transcardiac perfusion with 4% paraformaldehyde for neurogenesis assessment.

2.2. Fluoxetine treatment paradigm and plasma analysis

Fluoxetine hydrochloride (Sigma–Aldrich, St. Louis, MO) was provided for 35 days in drinking water containing 0.066% saccharin to mask any taste of the drug beginning at postnatal day (PD) 35. Male offspring from dams drinking arsenic or tap water were separated into three groups: those drinking 50 mg/L fluoxetine, 100 mg/L fluoxetine, and control (only 0.066% saccharin) as seen in Fig. 1A. Fluoxetine solutions were shielded from the light and changed every other day. Weight gain and fluoxetine ingestion were monitored weekly.

2.3. Predator odor exposure and stress response

Exposure to 2,3,5-trimethyl-3-thiazoline (TMT) (Contech, Victoria, BC, Canada; #300000368) was performed as previously described (Goggin et al., 2012). Animals were individually transferred to an exposure cage containing filter paper saturated with 100 μL of 3% (v/v) TMT for 10 min. Group-housed animals were exposed concurrently in parallel and returned to home cages for 20 min after the 10-min exposure. Several cohorts of animals were exposed to TMT, some of which were placed into the forced swim task 20 min after exposure, while others were rapidly decapitated for trunk blood collection using Safe-t-Fill EDTA capillary tubes. Samples were centrifuged at 1000 × *g* for 10 min at 4 °C. Plasma was removed and stored at −80 °C. Corticosterone (CORT) levels in plasma were determined using the DetectX Corticosterone EIA Kit (Arbor Assays, K014-H1) per manufacturer instructions with a 1:100 dilution of plasma prior to analysis. Optical density absorbance was determined by an Infinite M200 instrument and data were analyzed using 4PLC ReaderFit online software.

2.4. Open field

Adult male mice (PD70) were permitted to explore a novel open arena, measuring 40 cm × 40 cm × 35 cm and constructed of white Plexiglas, for 15 min for two consecutive days. Dim red overhead lighting was used along with white spotlights to illuminate the corners of the open field at 45–60 lumen and the center at 75–90 lumen. Total distance traveled was measured with the aid of an Ethovision video tracking system using the coordinates of the animal's center of mass image compared against a template of the arena dimensions. Locomotor activity in a novel environment was assessed the first day, and