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Maternal exposure to carbamazepine at environmental concentrations can cross intestinal and placental barriers

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ABSTRACT

Psychoactive pharmaceuticals have been found as teratogens at clinical dosage during pregnancy. These pharmaceuticals have also been detected in minute (ppb) concentrations in drinking water in the US, and are environmental contaminants that may be complicit in triggering neurological disorders in genetically susceptible individuals. Previous studies have determined that psychoactive pharmaceuticals (fluoxetine, venlafaxine and carbamazepine) at environmentally relevant concentrations enriched sets of genes regulating development and function of the nervous system in fathead minnows. Altered gene sets were also associated with potential neurological disorders, including autism spectrum disorders (ASD). Subsequent *in vitro* studies indicated that psychoactive pharmaceuticals altered ASD-associated synaptic protein expression and gene expression in human neuronal cells. However, it is unknown if environmentally relevant concentrations of these pharmaceuticals are able to cross biological barriers from mother to fetus, thus potentially posing risks to nervous system development. The main objective of this study was to test whether psychoactive pharmaceuticals (fluoxetine, venlafaxine, and carbamazepine) administered through the drinking water at environmental concentrations to pregnant mice could reach the brain of the developing embryo by crossing intestinal and placental barriers. We addressed this question by adding ^{2}H-isotope labeled pharmaceuticals to the drinking water of female mice for 20 days (10 pre-and 10 post–conception days), and quantifying ^{2}H-isotope enrichment signals in the dam liver and brain of developing embryos using isotope ratio mass spectrometry. Significant levels of ^{2}H enrichment was detected in the brain of embryos and livers of carbamazepine-treated mice but not in those of control dams, or for fluoxetine or venlafaxine application. These results provide the first evidence that carbamazepine in drinking water and at typical environmental concentrations is transmitted from mother to embryo. Our results, combined with previous evidence that carbamazepine may be associated with ASD in infants, warrant the closer examination of psychoactive pharmaceuticals in drinking water and their potential association with neurodevelopmental disorders.

1. Introduction

Multiple studies have identified a strong genetic component to the manifestation of neurodevelopmental disorders such as autism spectrum disorders (ASD) [1–3] that nonetheless can account only for a subset of diagnosed cases. In addition, the presence of individual low risk contributing susceptibility genes, or common variants [3] may not suffice as causal agents without the interaction with other environmental, epigenetic, or stochastic factors to cause ASD [4–6]. These findings suggest that a majority of cases may result from the presence of unknown environmental triggers in genetically susceptible individuals [1,5–7]. Additional studies have provided evidence that maternal environmental exposures (especially in the first trimester of pregnancy) play a role in the etiology of ASD [1,7]. These studies have added to a relatively smaller but

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expanding body of literature addressing the role of environmental contaminants in the etiology of neurodevelopmental disorders [1,3].

In industrialized countries, humans are exposed to nearly 3000 synthetic compounds through daily use and the environment [1,8]. For many of these compounds it remains poorly understood how their presence in water, air, and/or food affect human health [1,8] and to what extent they may contribute to neurological disorders in susceptible individuals [6,7]. Pharmaceuticals and personal care products (PPCPs) are some of the largest and most widely used classes of synthetic compounds. These products include commonly prescribed pharmaceuticals, phthalates in cosmetics, bis-phenol A (BPA) in plastics and other teratogenic chemicals [1]. Among PPCPs, our laboratory has been studying psychoactive pharmaceuticals (including fluoxetine, venlafaxine and carbamazepine) as potential neurological disorder-relevant contaminants that have been detected in the drinking water [8,9].

Psychoactive pharmaceuticals (PPs) are widely prescribed [9] and, following excretion from patients, found in water discharged from waste-water treatment plants (WWTP) [8,10]. The excreted products of PPs are metabolically active and have long half-lives [8,10]. The pharmaceuticals reach surface waters after inefficient filtration at WWTP and thereafter reach drinking water through ground-water or other supply routes [8,10].

We studied psychoactive pharmaceuticals such as fluoxetine, venlafaxine and carbamazepine, as they are among the most commonly prescribed antidepressants [9]. In addition, at clinical doses these drugs have demonstrated effects on the prevalence of autism spectrum disorders (ASDs) [9]. Clinical studies have reported that 1.8%–2.8% of women consume antidepressants during their pregnancy [11] and prenatal exposure to these antidepressants is linked to increased risk of ASD in infants [12]. Furthermore, at clinical doses, anti-epileptic drugs (AEDs), such as carbamazepine, have well-studied teratogenic effects [13]. A group of researchers examined infants exposed to carbamazepine during prenatal development and found them to have lower IQ scores and impaired cognitive function compared to non-exposed infants [14].

To determine if these PPs alter neurophysiology at concentrations found at WWTP, our lab carried out preliminary studies by treating juvenile fathead minnow fish with pharmaceuticals (fluoxetine, venlafaxine and carbamazepine) at environmentally relevant concentrations, which were 6–10 times higher than concentrations measured in drinking water throughout the US [8,9]. The increase was chosen to accommodate for the various byproducts and modified forms of each drug as they occur in the environment and to account for the cumulative effects of multiple drugs [8,9]. Using DNA microarray-based expression profiling of exposed fish brains, we found that PP-treated water significantly induced the expression of gene sets associated with neuronal growth, development, regulation, and neurological disorders (including idiopathic autism) [8,9].

Recent studies have found that *in utero* exposure to PPs like antipsychotics, antidepressants and benzodiazepines during pregnancy may result in the development of neurologic, respiratory, gastro-intestinal and autonomic abnormalities in newly born infants [11]. These pharmaceuticals can be grouped into selective serotonin reuptake inhibitors (SSRI), such as fluoxetine; serotonin-norepinephrine reuptake inhibitors (SNRI), such as venlafaxine; mood stabilizers, such as valproate and carbamazepine; and benzodiazepines, such as diazepam, temazepam and nitrazepam [11,15]. Studies from human subjects have reported the placental transfer of antidepressants (fluoxetine, sertraline, nortriptyline, and desmethyl clomipramine) to umbilical cord blood [16,17] when taken from mothers at clinical dosage, providing evidence that these antidepressants can cross the intestinal and placental barriers [16,17]. It is unknown, however, if these psychoactive pharmaceuticals can also cross intestinal and placental barriers when ingested at concentrations observed in drinking water, a question of relevance to all pregnancies where contaminated drinking water may be consumed.

In the present study, we hypothesized that PPs (fluoxetine, venlafaxine and carbamazepine) consumed by pregnant mothers at typical environmental concentrations would cross the intestinal and placental barriers, and reach the brain of the developing embryos. To determine this, we dissolved deuterium-labeled PP in the drinking water of pregnant mice and measured deuterium (^{2}H) enrichment in the developing embryonic brains and maternal livers. We found carbamazepine, but not fluoxetine or venlafaxine in both tissues confirming its passage through biological barriers during pregnancy.

2. Materials and methods

2.1. Ethics statement

All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Idaho State University and performed in accord with the NIH (National Institutes of Health) *Guide for the Care and Use of Laboratory Animals.*

2.2. Drug treatments

Fluoxetine D6-oxalate (FLX) [#F919; 98 atom (at) % ^{2}H], Carbamazepine (CBZ) [# DLM-2806-1.2; 98 at % ^{2}H], Venlafaxine (VNX) [#V009; 98 at % ^{2}H] and L-glutamic acid [# DLM-556-0.05; 98 at % ^{2}H] were obtained from Cambridge Isotope Laboratories Inc. (MA). The overall experimental design comprised five treatments: FLX (concentration = 10 μg/l; $n = 4$), VNX (50 μg/l; $n = 4$), CBZ (100 μg/l; $n = 4$), control (no treatment; n = 4) and negative control (contaminated with ^{2}H-labeled L-glutamine; $n = 1$). To examine the possibility of embryonic tissue contamination by maternal blood during dissection, we performed control experiments by bathing dissected embryos in phosphate buffer saline (PBS) containing 0.03 mg/ml ^{2}H-labeled L-glutamine solution followed by 7–8 washes with PBS. Subsequent measurements of embryonic tissue resulted in no detection of ^{2}H leading us to adopt the same method for experimental dissections.

2.3. Animal experiments

Female and male C57BL/6 mice (50 days old) were obtained from the Jackson Laboratory (Sacramento, CA) and placed on a standard chow diet for two weeks prior to experiments. Each experiment was ~20 days long. Starting at Day 1 and for the entire duration of the experiment, four female mice were given water containing isotope-labeled pharmaceuticals *ad libitum*. Water bottles were protected from light to avoid any photo-degradation of pharmaceuticals and the level of water was monitored daily confirming uniform consumption across test groups. On day 10, each female was housed with one male and examined for vaginal plug presence over the following five days. The day when a plug was observed was marked as embryonic day 0 (E0) of the pregnancy. On embryonic day 10 (E10), prior to the formation of the blood–brain barrier (BBB), pregnant mice were euthanized by CO_2 asphyxiation followed by cervical dislocation. In each dissection, we collected a lobe of the mother's liver and brains of all embryos. Each embryo was separated from extraembryonic membranes and microdissected under ice cold PBS to remove the brain. Using a dissecting microscope, we removed embryonic brains using a knife. Thus, we collected one maternal liver (a lobe) and five embryonic brains

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