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A study of the age-related effects of lactational atrazine exposure

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ABSTRACT

A growing number of reports have demonstrated that the widely-used herbicide Atrazine (ATR) can cause injury to dopamine (DA) neurons, but the exact mechanism remains unclear. In this study, we examined the effects of lactational ATR exposure in Sprague-Dawley rats on dopaminergic neuron health later in life.

Compared with control rats, rats exposed to ATR during a critical period of neural development showed decreased striatal DA content and increased rates of DA turnover. The expression of Monoamine oxidase (MAO), which is associated with DA degradation, was up-regulated, and the expression of Vesicular Monoamine Transporter 2 (VMAT2), which is associated with DA transport, was down-regulated. The expression of transcription factor Nuclear Receptor Related Factor 1 (Nurr1), which is associated with DA neuron development, was down-regulated. Increased age (6–12 months old) increased the statistical significance of the differences of the above indicators in the ATR-treated rats compared to the control rats ($P < 0.05$).

Taken together, our results indicate that ATR exposure during the critical neural development period causes a down-regulation of Nurr1, which in turn affects Nurr1 target genes, including MAO, VMAT2 and DAT, which are involved in DA degradation and transport. Reduced expression of these genes impairs the capacity for vesicular storage or reuptake of DA, causing decreased levels of striatal DA, which can ultimately lead to DA neuron injury. DA neuron injuries become more severe over time, which suggests that aging can synergistically promote the ATR-associated DA neuron injuries.

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1. Introduction

The use of atrazine (2-chloro-4-ethytlamino-6-isopropylamine-1,3,5-triazine; ATR) is widespread throughout agriculture; however, there are public health concerns regarding the effects of ATR on reproductive and developmental outcomes.

Recent studies in rodents showed that ATR treatment can alter locomotion[1], decrease striatal dopamine (DA) content and decrease the number of tyrosine hydroxylase-positive (TH+) neurons in the midbrain [2]. Long-term (1-year) dietary ATR exposure decreases striatal DA in rats and alters motor activity [3]. Short-term (14-d) oral ATR exposure induces hypoactivity, object recognition memory deficits, and anxiety-like behavior that are accompanied with altered serotonin (5-hydroxytryptamine) and DA homeostasis in the striatum in adult mice [4]. These findings indicate that rodent dopaminergic neurons may be a target of ATR exposure, causing a range of cellular, molecular and behavioral abnormalities.

The underlying pathology of Parkinson's disease (PD)-like symptoms is the dysfunction of dopaminergic neurons. Although most PD cases are diagnosed later in life, the pathological defects arise before the disease has progressed to the point at which it is diagnosed [5]. The long preclinical phase and links between environmental exposures and increased risk of PD have led to speculation that exposures to environmental toxicants in early life may enhance dopaminergic neurodegeneration or increase the vulnerability of the dopamine system later in adulthood [6].

While the epidemiological evidence of the neurodevelopmental toxicity of pesticide exposure during pregnancy is growing, occupational exposure to pesticides during pregnancy is associated with low gestational age and a greater risk of prematurity and low birth weight [7]. Environmental exposure to ATR during pregnancy has been associated with various adverse birth outcomes, including preterm birth. Striatal structural abnormalities are a common pathology in the brains of preterm infants [6], suggesting a possible epidemiological correlate between developmental ATR overexposure and basal ganglia disorders.

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Several studies have indicated that the developing nervous system is particularly sensitive to ATR and that some effects of ATR exposure are only observed in adulthood. For example, embryonic ATR exposure decreases 5-hydroxyindoleacetic acid (5-HIAA) and serotonin turnover in adult female zebrafish brain tissue [8], causing dysfunction and molecular alterations in adult zebrafish and morphological alterations in their offspring [9]. Gestational and/or lactational exposure to ATR was found to delay vaginal opening and mammary gland development [10]. Oral exposure to ATR from gestational day 14 to postnatal day (PND) 21 alters motor activity in juvenile offspring and causes extensive neurodegenerative alterations in cortical, hippocampal, hypothalamic, and striatal areas of adult offspring [11]. During embryonic development, organisms exhibit a high level of developmental plasticity, allowing for alterations in their genetic landscape in response to the surrounding environment, which can ultimately result in a broad range of adult phenotypes [12].

However, the mechanism of injury to dopaminergic neurons induced by developmental ATR exposure is still incomplete; therefore, our study aimed to determine the effects of ATR exposure during lactation on dopamine-relevant neurochemistry in juvenile, adults and aged offspring.

2. Materials and methods

2.1. Chemicals and reagents

ATR (CAS Registry Number: 1912-24-9; 98% purity) was obtained from Chem Services (West Chester, PA, USA). DA, dihydroxy-phenyl acetic Acid (DOPAC) and homovanillic acid (HVA) were purchased from Sigma (St. Louis, MO, USA).

2.2. Animals and treatment

Forty nulliparous female (220–250 g) and 20 male (300–320 g) Sprague Dawley rats were purchased from Vital River Laboratories (Peking, China). The animals were treated in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institutes of Health. All efforts were made to minimize the number of animals used in the experiments and their suffering.

After acclimatization for 1 week, female rats were housed in pairs in standard stainless steel cages until mating. For breeding, two nulliparous female rats were placed with one male overnight and removed the next morning, and gestation was identified by the appearance of a vaginal plug. The pregnant rats were housed alone in standard polyethylene cages, with wood shavings as bedding.

Thirty pregnant rats were selected and randomly divided into three groups (ten rats per group). The day the pups were born was referred to as PND 0. Dopamine system development starts from gestational day 6 in rodents and the dopaminergic neurogenesis peaks around day E12.5 and declines thereafter. Hereafter, until the first postnatal weeks, the dopaminergic neurons start extending axonal outgrowths towards their target projection areas within the striatum and cortex [13]. Our previous study found that gestational exposure to ATR can result in significant decrease of concentration of DA in the striatum [14]. In this work we want to know the toxicity effects on dopaminergic neurons in offspring exposure to ATR during lactation period. So the exposure duration start from PND 1. Dams received daily ATR or vehicle treatment by 10 μL/g bodyweight oral gavage starting on PND 1 until the pups were weaned on PND 23. The ATR doses used were 0, 25 and 50 mg/kg/day (3% starch solution, vehicle control). These doses were based on the concentrations used in previous studies [4,15,16] and similar to 34.5 mg/kg/day, which is used to calculate the lowest observed adverse effect level [17]. To minimize the stress-related effects in any particular group the oral gavage was performed at approximately the same time in the morning each day.

On PND 23, we divided all pups from the 10 pregnant rats in each group according to sex, and then used the stratified random sampling method to select the weanlings, yielding a total of 30 weaning pups from each treatment group (15 females and 15 males). These pups were separated by group and sex into separate cages and housed for the duration of the study; the remaining littermates were euthanized with chloral hydrate (300 mg/kg) [18].

Throughout the experiment the animals were given purified water (A10; Millipore, Bedford, MA, USA) *ad libitum*. Pregnant and lactating females were given Rat Chow 16073111, while weanling and adult rats were given Rat Chow 16033231 (Beijing Keao Xieli Feed Co. Ltd., Beijing, China; license number SCXK(jing) 2014-0010) *ad libitum*. The animal rooms were maintained at a constant light/dark cycle (on at 06:00 h, off at 18:00 h), temperature of 22 ± 2 °C and relative humidity of 50 ± 15%.

Animals were observed daily for changes in appearance, behavior and mortality, and food consumption and body weight were monitored regularly. All parental animals were sacrificed after completing lactation. Litter weight and litter size was recorded at birth; pup survival was recorded on PND 21.

At 3, 6 and 12 months, 10 offspring (five males and females) in the control and ATR-treated groups were euthanized by chloral hydrate (300 mg/kg) [18]. Brains were rapidly removed, rinsed with ice-cold saline, and further dissected to remove the midbrain and the entire striatum of the bilateral side of the brain. The striatum was weighed, and then all tissues were immediately frozen and stored at −80 °C until further processing. Dopaminergic neurons are mainly concentrated in the midbrain, of which the development and differentiation are regulated by genes in this section [13]. Striatum is the main projection field of dopaminergic neurons, which become the main sites of DA transmitter secretion [13]. We measured protein and mRNA levels using midbrain samples and DA levels using striatum samples.

2.3. High-performance liquid chromatography fluorescence (HPLC-FL) detection of DA, DOPAC and HVA

The DA, DOPAC and HVA concentrations in the striatum were assessed using an HPLC system with a fluorescence detector [19]. The striatum from the rats in each group were homogenized in 0.1 M perchloric acid and centrifuged at $10{,}000 \times g$ for 20 min at 4 °C. The homogenized samples were then filtered using a 0.2 μm cellulose membrane. The supernatants were then analyzed to determine DA, DOPAC and HVA content, then the samples were injected into a chromatograph equipped with a fluorescence detector (Agilent, Santa Clara, CA, USA) and a COSMOSIL C18 Column (5 μm, 4.6 mm × 250 mm; Nacalai, Kyoto, Japan). The mobile phase consisted of trisodium citrate (20 mM) and EDTA (0.1 mM); the pH of the mobile phase was adjusted to 5.1 by adding glacial acetic acid.

Samples were separated at room temperature with a flow rate of 1.0 mL/min. The HPLC detector was set at an excitation wavelength of 285 nm and an emission wavelength of 333 nm. Data were quantified using the area under the peaks and external standards. Quantification was verified using calibration curves obtained from individual monoamine standards as reference.

DA turnover rate is a value that reflects the speed of dopamine uptake. The formula is as follows:

$$DAturnoverrate = \frac{DOPAC + HVA}{DA}$$

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