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Effect of gestational exposure to arsenic on puberty in offspring female mice

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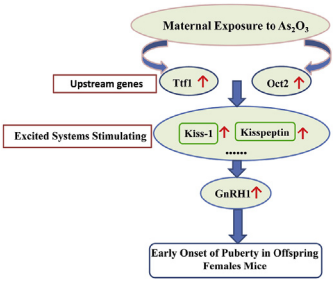
HIGHLIGHTS

- The vaginal opening time was significantly advanced in offspring females exposed to As.
- As gestational exposure significantly increased LH level at pubertal age in offspring females in utero.
- As significantly altered the mRNA and protein expressions of the puberty related genes in offspring females at pubertal age..

GRAPHICAL ABSTRACT



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ABSTRACT

We examined the vaginal opening day, ovary and uterus organ coefficient, reproductive hormone levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), mRNA and protein expression levels of kiss-1, hypothalamus gonadotrophin releasing hormone 1 (GnRH1), organic cation transporters 2 (Oct2) and transcription termination factor 1 (Ttf1) in different pubertal ages [late lactation (18 days), pre-puberty (21–22 days), puberty (23–27 days; with respect to vaginal opening) and maturity (65 days)] of offspring females, to evaluate the effect of arsenic (As) on puberty initiation after maternal exposure to As at different concentration [0, 0.15, 1.5 and 15 mg/L As(III)] during gestational period. The results showed that the vaginal opening time was significantly advanced in utero in mice exposed to As compared to the control. The hormone level of LH was significantly increased in the mice treated with 15 mg/L of As(III) at puberty compared to the control. During puberty, the mRNA expression levels of kiss-1, GnRH1, Oct2 and Ttf1 in the hypothalamus were significantly increased in the group treated with 15 mg/L of As(III) compared to the control. The protein expressions of Kisspeptin, GnRH1, Oct2 and Ttf1 in the hypothalamus were significantly increased in the pubertal females, while Oct2 and Ttf1 expression levels were significantly decreased in the matured females compared to the control, which is in line with the transcriptional changes of related mRNA expressions. In brief, this study demonstrated that maternal exposure to As during gestational period could result in early onset of puberty in offspring females.

1. Introduction

As, a semi-metal and environmental endocrine disruptor, is naturally found in the environment, and considered a common contaminant in drinking water and crops (Reilly et al., 2014; Kirkley

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et al., 2018). Recent studies reported humans are exposed to As through occupational or non-occupational ways, and that As can cross placental and blood-brain barriers (Hirner and Rettenmeier, 2010; Luo et al., 2013). This implies that gestational exposure to As would have an adverse effect on the development of offspring. Recent studies proved that As influences offspring in hormone receptor synthesis, decomposition and activity in vivo (Caldwell et al., 2015). Rodriguez et al. demonstrated that As exposure has affected the sexual maturation time of female offspring (Reilly et al., 2014; Rodriguez et al., 2016). However, results in the literatures are contradictory, the relation between the in utero As exposure and the pubertal development was still unclear. Parodi et al. showed that in utero exposure to As contributed to the early vaginal opening in the female mice. Also, early onset of vaginal opening was reported in female CD-1 mice in utero exposure to As (Parodi et al., 2014; Rodriguez et al., 2016). However, delayed puberty was reported in rats exposed to 3 ppm As from gestation to 4 months of age (Dávila-Esquedaa et al., 2012).

Puberty is the intricacy of maturational occurrence, a developmental process that features physical and psychosocial aspects. Adultness is the achievement of sexual and somatic maturation, and reproductive competence. Unnatural pubertal timing can influence physical and psychosocial well-being of children (Klein et al., 2017). Recently, Li et al. reported that number of sufferers with sexual precocity has significantly increased, and in females it is 10 times greater than males (Li et al., 2014). Sexual precocity in girls may lead to physical and psychological problems, such as increased behavioral disorders, breast cancer risk, polycystic ovarian syndrome, reproductive system neoplasms and so on (Pasquino et al., 1999; Golub et al., 2008; Kim, 2015). Hence, it is a question of importance to be answered, for individuals and society.

Kiss1 and G protein-coupled receptor 54 (GPR54) can define the correct timing of puberty expressed in diverse cellular subsets of the hypothalamus. The Kiss-1 gene encodes, a cluster of biologically active peptides commonly known as Kisspeptin, which plays a primary regulatory role in puberty onset and reproductive function (Tenasempere, 2010). Other genes involved in the transcriptional regulation during pubertal processes, includes the POU-domain gene of Oct2, and the homeodomain gene of Ttf1/NKX2.1. The POU-domain is regarded as the key regulatory gene for sexual maturation which is widely expressed in pubertal mammalian brains. It has been put forward that Oct2, Ttf1 and EAP1 are upstream genes that control the release of subordinate genes distinctively expressed in neurons and glial cells, which are required for the neuron-to-neuron and glia-to-neuron administration of hypothalamus gonadotrophin releasing hormone (GnRH) excretion during puberty (Ojeda et al., 1999, 2010).

However, it is still unclear how As exposure affects these puberty related genes. In the present study, in order to assess the effect of As exposure in utero on puberty initiation (advanced or delayed) of offspring females after maternal exposure to As during gestational period, we examined vaginal opening day firstly, and then evaluated the levels of mRNA and protein of Kiss-1, GnRH1, Ttf1 and Oct2 in offspring females at different pubertal ages [late lactation (18 days), pre-puberty (21–22 days), puberty (23–27 days; with respect to Vaginal opening) and maturity (65 days)]. Also, body weight changes, ovary and uterus organ coefficients, and reproductive hormone levels (LH and FSH) were examined.

2. Material and methods

2.1. Animals and treatments

Forty-eight 8-week old, healthy female ICR mice, were obtained from Experimental Animal Center in Academy of Military Medical Sciences. Before the experiment, mice were kept in plastic cages with wood shavings, in temperature-controlled rooms under controlled lighting (12 h light: 12 h dark) to acclimate to the environment. Deionized water and commercial rodent pellets were freely provided all the time. All animal procedures were performed in compliance with the regulations and guidelines of international ethics committee on animal welfare.

After one-week of acclimatization, the ICR mice were randomly divided into four groups ($n = 12$ per group): control group (drinking deionized water) and three As treated groups [concentration of As(III) in deionized water: 0.15 mg/L, 1.5 mg/L, 15 mg/L]. Maternal mice on an average daily drink about 10 mL/day, that resulted in intake of 1.5 μg, 15 μg and 150 μg per female mouse per day in low, medium and high groups respectively. The day vaginal plug was found, regarded as embryonic day 0 (E0), and then the pregnant females were housed separately in a plastic cage until offspring were born. Then all the maternal mice were treated with deionized water throughout the whole lactation period.

Female pups were checked at 8:00 a.m. daily for vaginal opening starting from 18-day of age. Onset of puberty in female mice is characterized by becoming pink, moist, and swollen at the vaginal opening, and it can also be determined based on vaginal cytology via vaginal smear (Fig. 1). Twelve offspring females from each group were sacrificed using cervical vertebrae luxation according to animal ethical standards at late lactation (18 days), pre-puberty (21–22 days), puberty (23–27 days, vaginal opening) and matured (65 days) stages, and their serum and hypothalamus were collected for subsequent analysis.

2.2. Organ coefficient

The ovaries and uterus from each group were carefully removed and weighed. The percentage of organ coefficient was calculated according to the formulae: Organ coefficient = [Organ wet weight (g)/Body weight (g)] ×100%.

2.3. Serum preparation and ELISA

Following sacrifice, blood was collected from female mice and serum was isolated by centrifugation at 3000 rpm for 15 min at 4 °C, and frozen at −80 °C to measure the levels of kisspeptin, GnRH1, LH and FSH by ELISA method, based on the manufacturer's instructions. In brief, 100 μL standard and samples were added to each well and incubated for 90 min at 37 °C. Meanwhile, blank wells were set at the same time. Then the liquid was removed, and 100 μL of Biotinylated Detection Ab was added and incubated for 1 h at 37 °C. After being washed 3 times, 100 μL HRP Conjugate was added to the wells except for the blank wells, and incubated for 30 min at 37 °C. Again, after being washed 5 times, 90 μL substrate reagent was added, and the wells were incubated for 15 min at 37 °C. Then 50 μL stop solution was added to terminate the reactions, and the absorbance (OD) was read at 450 nm immediately by the Pan-wavelength Multimode Reader (Elabscience Biotechnology Co., Ltd, Wuhan, China). Optical density was calculated by CurveExpert1.4 software.

2.4. Total RNA extractions and real-time PCR (RT-PCR)

Total RNA was extracted using Trizol Reagent (Takara Biological Engineering Company, Dalian, China) from the hypothalamus in accordance with the manufacturer's instructions and digested by RNase-free DNase I (Promega Madison, WI, USA) to eliminate genomic DNA contamination. Then, RNA quality was examined by Nano Drop ND-1000 Spectrophotometer (Nano-Drop, USA) and agarose gel electrophoresis. The reverse transcription (RT) tests