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Hormonal changes and folliculogenesis in female offspring of rats exposed to cadmium during gestation and lactation☆

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ABSTRACT

It has been suggested that the toxic effects of cadmium (Cd) may disrupt ovarian and uterine functions in adults. However, Cd exposure during gestation and lactation and its effects on the reproductive development in female offspring is still not clear, and the mechanisms underlying exposure toxicology remain mostly unexplored. To investigate how Cd exposure of female rats (F0) during gestation and lactation affects the reproductive development of their female offspring, we studied the steroidogenesis, folliculogenesis, puberty onset, and litter size of the first (F1) and second (F2) filial generations following F0 female rats which had been exposed to $CdCl_2$. The mechanisms related to the early onset of puberty induced by such exposure in female offspring were explored. Maternal exposure to Cd dramatically increased the biosynthesis of steroid hormones in F1 female offspring by the activation of cAMP/PKA pathway and up-regulated expression of steroidogenesis related proteins such as StAR, CYP11A1, 3β-HSD and CYP19A1. The high levels of steroid hormones contributed to an early puberty onset, promoted the differentiation and maturation of follicles, and led to the proliferation of endometrium that resulted in a uterus weight gain. The increased number of antral follicles eventually caused a big litter size. Despite of being free from additional Cd exposure, the levels of CYP11A1 and CYP19A1 in the ovaries of F2 female rats were also high, which resulted in a high concentration of serum progesterone. These results suggested that hormonal changes induced by exposure to Cd in utero might have a lasting effect beyond the first generation. These findings may help to better understand the origin of female sexual dysfunction in the developmental stages in general.

1. Introduction

The heavy metal cadmium (Cd) is widely dispersed as an environmental toxicant. It has a particularly long half-life when accumulated in the body, usually leading to a life long suffering for the patient (Jarup et al., 1998). The metal has been used widely in many occupations, including electroplating, welding, mining, Ni-Cd batteries and even plastic making. Exposure to Cd increases susceptibility to tumorigenesis such as lung, uterus, and breast carcinomas in females (Person et al., 2013; Gallagher et al., 2010). Increasing evidence indicates that Cd can affect female reproductive functions, resulting in infertility, miscarriage, premature birth and infant mortality (Wu et al., 2008). As an endocrine disrupting chemical, Cd can impact sex steroid hormone production, the development of uterus and the quantity of ovarian follicles in adult female rats (Amutha and Subramanian, 2013; Liu et al., 2010). However, the influence of Cd exposure during gestation and lactation on the reproductive development of female offspring is still not evident, and the mechanistic chain of events following such exposure has not been fully explored.

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Production of estrogen starts with the synthesis of pregnenolone from cholesterol, which is transported by steroidogenic acute regulatory protein (StAR), and catalyzed by the cytochrome P450 side chain cleavage enzyme (P450scc/CYP11A1). Pregnenolone is then converted to progesterone (P4) by 3-beta-hydroxysteroid dehydrogenase (3β-HSD) in both thecal and granulosa cells. P4 is next converted to androstenedione via cytochrome P450 17α-hydroxylase (P45017α) and 17-beta-hydroxysteroid dehydrogenase (17β-HSD) in thecal cells during the follicular phase. Androstenedione diffuses across the basal lamina into the granulosa cells. Follicle-stimulating hormone (FSH) stimulates the expression of aromatase cytochrome P450 (CYP19A1) and 17β-HSD in the granulosa cells by a cAMP-mediated mechanism. The granulosa cells can then metabolize androstenedione to estradiol (E2) (Cui et al., 2013; Magoffin, 2005). CYP19A1 is the rate-limiting enzyme, aromatizes androgens to E2 in granulose cells (Pan et al., 2012). In the synthesis of pregnenolone, ERK1/2 phosphorylation is triggered by a cAMP/cAMP-PKA-dependent mechanism, and StAR is a substrate of ERK1/2. Mitochondrial ERK1/2, a part of the multimeric protein kinase complex, regulates cholesterol transport (Duarte et al., 2014). cAMP/PKA is involved downstream of dihydrolipoamide dehydrogenase (DLD), which activates phosphorylation of ERK1/2 to initiate the steroid biosynthesis (Ji et al., 2015; Yan et al., 2008).

Earlier studies have shown that Cd is an endocrine disruptor, affecting the ovarian functions in adults including steroidogenesis (Amutha and Subramanian, 2013). Although many of such studies have focused on delineating the toxicity of Cd to the reproductive system of females, a few have focused on studying the reproductive development in the female offspring of parental generation exposed to Cd. Exposure to Cd in utero has been shown to accelerate puberty onset as determined by an earlier than usual timing of vaginal opening (VO), although no mechanism underlying such change has been disclosed (Johnson et al., 2003). The exposure also led to an increase of the parenchymal area of mammary gland and the number of terminal end buds (Johnson et al., 2003), which might be due to the increased level of E2 in female offspring (Davis et al., 2013). On the other hand, other studies have found that there was no adverse effects on the physical and sexual development in the pups other than delaying the development of offspring (Luo et al., 2015). There is no consensus on the outcome of Cd exposure on the sexual development of female offspring, and the mechanism by which Cd exerts its influence is not clear. What is more, there is no report in the literature on whether maternal exposure to Cd affects the reproductive ability of the first filial generation (F1) of females, nor any report on what the impact might be, if any, on the sexual development of the second filial generation (F2) that are free from any additional Cd exposure. Cd levels that are increased in maternal blood and accumulated in placenta have the potential to affect the foetal genome via epigenetic modifications such as DNA methylation (Dharmadasa et al., 2017). The possibility that heritable epigenetic variation may lead to hormonal changes for later generation intrigued us, and prompted us to extend our study on the F2 generation which is without maternal Cd exposure.

To investigate how continuous Cd exposure in female rats (F0) during gestation and lactation may affect the reproductive development of their female offspring, we studied the steroidogenesis, folliculogenesis, onset of puberty and litter size in the first (F1) and second filial (F2) generations following the F0 female rat exposure to the metal. Mechanism leading to the early onset of puberty of the offspring induced by such exposure was explored. These studies might provide more insight and better understanding of the pathophysiological process of precocious puberty in humans.

2. Materials and methods

2.1. Animals and treatment

Adult male and female Sprague-Dawley rats weighing 200 ± 20 g were purchased from the Medical Experimental Animal Center of Guangdong Province. All rats were given standard food and tap water ad libitum, kept under conditions of controlled temperature (24 ± 1 °C), humidity (50–60%) and a light/darkness cycle of 12/12 h (light on at 8:00 a.m.). After acclimatization for a week, male and female rats (F0) were mated to obtain F1 generation. F0 female rats were separated after positive identification of a vaginal sperm plug, and the day was defined as designation of gestational day zero (GD 0). At GD 0, pregnant rats were housed individually in plastic cages and randomly divided into three groups. Female animals in the control group received water by gavage and other animals in the experimental groups received cadmium chloride ($CdCl_2$; Sigma, St. Louis, MO, USA) solution by gavage (1 mg/kg body weight defined as Cd1 and 5 mg/kg body weight defined as Cd5 corresponding to 1/90, 1/18 of LD 50) during gestation and lactation periods (Ronco et al., 2011; Samuel et al., 2011). An average oral lethal dose (LD50) value for $CdCl_2$ in rats was reported as 88 mg/kg body weight (Nasiadek et al., 2014). The day of parturition was designated as postnatal day zero (PND 0). Cd was administered from GD 0 to PND 21. In the administration period, dams kept survival; mortality rate was zero in pubs without deformity. Some offspring were weaned at PND 21 and housed in unisexual groups in litter-only stations with free access to lab diet and drinking water. Rats were monitored daily for VO and first estrous (markers of puberty onset) from PND 21 to 45. On the day of VO, vaginal fluid was collected to detect the day of first estrus (predominance of cornified epithelial cells). At PND 21, 35 and 56, F1 female rats were weighed and sacrificed by anesthesia (pentobarbital sodium, 60 mg/kg), and the blood samples were collected by intracardiac puncture. The periods under investigation covered female rats' juvenile (PND 21-35), peripubertal (PND 35-55) periods and adulthood (PND 56-) in order to ensure an accurate assessment of the effects on the growth and maturation of the reproductive system (Kwak et al., 2017). At PND 56, vaginal smears were conducted to evaluate the stage of the estrous cycle (Borges et al., 2017). The levels of E2 and P4 were most stable during diestrus (Butcher et al., 1974), so female rats on diestrus were selected to collect the blood and tissue samples for detection. F1 female rats were allowed to mate with normal male rats to obtain the F2 generation, and F2 female rats were subjected to the same treatment conditions as F1 female rats. All experiments were conducted according to the guidelines for animal care and use of China and were approved by the animal ethics committee of the Chinese Academy of Medical Science.

2.2. Histopathological examination of uterus and ovary

Ovaries and uterus were fixed in 4% paraformaldehyde and embedded in paraffin, and sections (5 μm) were stained with hematoxylin-eosin for histological observation. According to the classification of primordial, primary, secondary follicles (Cheng et al., 2002), the number of follicles was counted in 8 randomly selected fields per slide of ovary.

2.3. Steroid hormone detection by radioimmunoassay

Blood samples were centrifuged at 3000×g/min for 10 min in a refrigerated centrifuge at 4 °C to separate plasma for hormone analysis. Concentration of E2 and P4 in serum was measured by I^{125} RIA Kits (Beijing North Institute of Biological Technology, Beijing,