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Exposure of preimplantation embryos to low-dose bisphenol A impairs testes development and suppresses histone acetylation of *StAR* promoter to reduce production of testosterone in mice

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ABSTRACT

Previous studies have shown that bisphenol A (BPA) is a potential endocrine disruptor and testicular toxicant. The present study focused on exploring the impact of exposure to low dose of BPA on male reproductive development during the early embryo stage and the underlying mechanisms. BPA (20 μg/kg/day) was orally administered to female mice on days 1–5 of gestation. The male offspring were euthanized at PND10, 20, 24, 35 or PND50. We found that the mice exposed to BPA before implantation (BPA-mice) displayed retardation of testicular development with reduction of testosterone level. The diameter and epithelium height of seminiferous tubules were reduced in BPA-mice at PND35. The numbers of spermatogenic cells at different stages were significantly reduced in BPA-mice at PND50. BPA-mice showed a persistent reduction in serum and testicular testosterone levels starting from PND24, whereas *GnRH* mRNA was significantly increased at PND35 and PND50. The expressions of testicular *StAR* and *P450scc* in BPA-mice also decreased relative to those of the controls at PND35 and PND50. Further analysis found that the levels of histone H3 and H3K14 acetylation (Ac-H3 and H3K14ac) in the promoter of *StAR* were decreased relative to those of control mice, whereas the level of Ac-H3 in the promoter of *P450scc* was not significantly different between the groups. These results provide evidence that exposure to BPA in preimplantation embryo retards the development of testes by reducing histone acetylation of the *StAR* promoter to disrupt the testicular testosterone synthesis.

1. Introduction

Bisphenol A (BPA), an environmental estrogenic chemical, is widely used in the production of polycarbonate plastics and epoxy resins for food containers, can linings, dental sealants, and baby bottles (Vandenberg et al., 2009; Geens et al., 2011). BPA has recently been the subject of intense public and scientific scrutiny due to increasing evidence that this compound poses a potential health risk (Vandenberg et al., 2013). There are many studies examining developmental BPA exposure in laboratory animals (Goodman et al., 2006; Palanza et al., 2008; Richter et al., 2007), but these have often generated inconsistent results. Some early studies have reported that low doses of BPA have minimal or no adverse effects in mice and rats (Cagen et al., 1999; Nagao et al., 2002; Ferguson et al., 2011). However, most agree that perinatal or neonatal exposure to BPA, even at doses lower than the "safe" exposure limit for humans, can bring about a high incidence of male reproductive and endocrine disorders in later life, such as cryptorchidism, decreased sperm count and fertility, and abnormal hormonal output in male rodents (Bai et al., 2011; Chapin et al., 2008; Richter et al., 2007). To date, detailed studies describing the effects of exposure to low-dose BPA in the early embryo on the development of the male reproductive system are lacking.

Male reproductive development is mainly regulated by testosterone, which stimulates the development of testes and reproductive tract at puberty and supports spermatogenesis and fertility

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in adulthood. Testosterone production is initiated through cholesterol transport in Leydig cells by the steroidogenic acute regulatory protein (StAR). Subsequently, genes directly responsible for testosterone synthesis include cytochrome P450 side chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17α-hydroxy/C17-20 lyase (P450c17), and 17β-hydroxysteroid dehydrogenase (17β-HSD) (Stocco, 2001; Payne and Hales, 2004). These steroidogenic enzymes are the key determinants of steroid biosynthesis and are vulnerable to the effects of environmental factors, such as BPA (Lavoie and King, 2009; Kim et al., 2011; Zhang et al., 2011, 2014a,b). Perinatal and postnatal BPA exposure has been reported to suppress the synthesis of testosterone by decreasing the expression of testicular steroidogenic enzymes P450scc and P450c17 (Xi et al., 2011). Akingbemi et al. (2004) found that oral administration of BPA (2.4 μg/kg/day) for 14 days inhibited testosterone production from Leydig cells in vitro by decreasing the expression of steroidogenic enzyme 17α-hydroxylase/17–20 lyase. It has remained unclear, however, whether exposure to BPA in the preimplantation embryo decreases testosterone by affecting the expressions of steroidogenic enzymes and whether these toxic effects are transient or permanent.

Preimplantation embryo development is regulated by epigenetic mechanisms as well as genetics. Epigenetics, such as DNA methylation and histone acetylation, refers to a stable change in gene expression potential without alteration of the DNA sequence (Berger et al., 2009). The epigenome is highly susceptible to environmental factors during embryogenesis because the DNA synthetic rate is high, and the precise patterns of DNA methylation and chromatin structure are set up at this stage. Additionally, if postfertilization epigenetic reprogramming is disturbed in germ cells, it may have the potential to affect imprinting and the epigenome in later life (Liu et al., 2008; Singh and Li, 2012). Therefore, it has been proposed that exposure to BPA in the early embryo may interfere with epigenetic programming, resulting in adverse developmental effects, and increasing the risk of adult-onset disease as a consequence.

Thus, the objectives of the present study were to dissect the effects of preimplantation exposure to BPA on male reproductive development and the underlying mechanisms. Testicular development, levels of reproductive hormones and expressions of testicular steroidogenic enzymes were investigated at various developmental stages following exposure to BPA. Our results indicate that preimplantation exposure to low-dose BPA can retard testicular development due to reduced testosterone levels and decreased steroidogenic enzyme expression that persist into adulthood. We also analyzed the epigenetic mechanisms involved in the effect of BPA on male reproductive development.

2. Materials and methods

2.1. Experimental animals

The present study was approved by the Animal Care and Ethical Committee of Nanjing Medical University. All procedures were in accordance with the guidelines of the Institute for Laboratory Animal Research of Nanjing Medical University. Twelve-week-old ICR mice (Oriental Bio Service, Inc., Nanjing, China), weighing~30 ± 2 g, were used at the beginning of this study. Animal rooms were maintained on a 12:12 light–dark cycle starting at 7:00 AM and were kept at a temperature of 22–23 °C. The animals were permitted free access to food and water ad libitum.

2.2. Preparation of the mouse model

Adult female mice were housed between cages of breeder males for a minimum of 1 week after arrival. Vaginal cytology was examined daily to detect their estrous cyclicity. Mice with two consecutive regular 4-d estrous cycles were used to mate with adult males. Adult male mice were placed in the female cages, and the morning on which a vaginal plug was observed was designated as the first day (D1) of pregnancy. BPA (99% purity; Sigma–Aldrich, Inc., St. Louis, MO) was dissolved in dimethylsulfoxide, and then diluted with olive oil (Bai et al., 2011; Chen et al., 2014). Dams were weighed, and BPA at 20 μg/kg body weight per day was orally administered from D1 to D5 in the evenings. Control mice received the same volume of vehicle. The dose was selected because a previous study had shown that the oral administration of BPA at 20 μg/kg body weight per day (from gestational days 11–17) significantly decreased the efficiency of daily sperm production in mice (vom Saal et al., 1998). In addition, the dose used in this study was below the U.S. EPA daily oral reference dose and the U.S. Food and Drug Administration acceptable daily intake of 50 μg/kg body weight per day (U.S. EPA, 1993).

No significant difference was found in litter size between control and BPA-treated mice. On PND4, litters were culled to 10 pups per litter, retaining the maximal number of males per litter. To minimize litter effects, all pups in a treatment group were pooled together, separated by sex, and then fostered to moms of the same treatment group. On PND21, the male offspring were weaned, transferred to plastic hanging cages (two to four per cage) and were permitted free access to food and tap water. The female offspring were used in other experiments. The weights of the male offspring were monitored frequently. During the treatment with BPA, dams did not exhibit weight loss or abnormal behaviors. Male pups from each litter were euthanized at PND10, 20, 24, 35, and PND50, respectively, and their testes were removed and weighed.

2.3. Histological examination

Testicular tissues from PND35 and PND50 were fixed in Bouin's fluid, followed by dehydration through a gradient series of alcohol, clearing in xylene and embedding in paraffin. Sections (5 μm thick) were rehydrated, and stained with hematoxylin and eosin (HE). Testicular morphology was examined with a conventional light microscope (Olympus DP70, Japan) and a 40/100 × objective. The tubular diameter and the height of the seminiferous tubule epithelium were measured in PND35 mice. At least 100 tubular profiles that were round or nearly round were chosen randomly and measured for each mouse. The epithelium height was obtained in the same tubules utilized to determine tubular diameter.

Sertoli cells in PND35 mice and germ cells in PND50 mice at stage VII-VIII of the seminiferous cycle, including type-A spermatogonia (Asg), preleptotene spermatocytes (pLSc), mid-pachytene spermatocytes (mPSc) and step 7 spermatids (7Sd) in round seminiferous tubules per testicle, were counted, in 100 round, randomly selected tubular cross-sections (Leblond and Clermont, 1952; Clermont and Morgentaler, 1955). The nuclei of Sertoli cells were differentiated from germ cells by their irregular shape, paler chromatin pattern, and a single nucleolus. Type A spermatogonia and preleptotene spermatocytes are mainly distributed on the basal side of seminiferous tubule (outfield). Type A spermatogonia have diffuse nucleus with fine-granulated chromatin. The preleptotene spermatocytes are characterized by thread-like clumps of chromatin. Mid-pachytene spermatocytes and step 7 spermatids lie close to the side of tubule lumen (infield). The midpachytene spermatocytes are characterized by large nuclei and mottled chromatin. The small step 7 spermatids lack heterochromatin.