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Neonatal exposure of male rats to Bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis

Smita Salian, Tanvi Doshi, Geeta Vanage*

National Center for Preclinical Reproductive and Genetic Toxicology, National Institute for Research in Reproductive Health (ICMR), JM Street, Parel, Mumbai 400 012, Maharashtra, India

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ABSTRACT

Background: Sertoli cell junctional proteins (SCJP) (viz. adhesion, gap and tight junctions) are important for spermatogenesis and perturbations in expression of these proteins are associated with impairments in process of sperm production. Bisphenol A (BPA) is an endocrine disrupter that has been associated with impaired spermatogenesis. However the mechanistic basis of impaired spermatogenesis is unknown, whether BPA is a Sertoli cell toxicant has not yet been fully investigated.
Objectives: The present study was undertaken to decipher the effects of neonatal exposure of male rats to BPA on fertility and its effect on the testicular expression of SCJP during development.
Methods: Neonatal male rats were s.c. injected with BPA at doses ranging from 0.6 to 10 μg/rat (100–1600 μg/kg bw of BPA) on post-natal days (PNDs) 1–5, and controls received vehicle. Diethylstilbestrol (DES) was used as a positive control. Male fertility was assessed during adulthood and the lowest dose of BPA that was most effective at impairing fertility was determined. Immunohistochemical localization for Connexin 43 (Cx-43, gap junctional), Zona Occludin-1 (ZO-1, tight junctions) and N-cadherin (adherens junction) was carried out on testicular tissue sections obtained from PNDs 15, 30, 45 and 90 of rats exposed to lowest dose of BPA that impaired fertility.
Results: Females mated with male rats that were exposed neonatally to various concentrations of BPA showed a significant increase in post-implantation loss and a decrease in litter size. There were significant changes in sperm count along with hormonal imbalances in the rats exposed neonatally to BPA. The 2.4 μg dose (400 μg/kg bw) of BPA was determined as the lowest dose that was capable of impairing male fertility. A significant reduction in the expression of Cx-43 (PND 45 and 90) and increases in the expression of N-cadherin (PND 45 and 90) and ZO-1 (PND 90) were observed in the testes of rats exposed neonatally to effective dose of BPA. Interestingly, there was an altered expression pattern of Cx43 amongst the sloughed cells in the testes of the experimental rats as compared to controls.
Conclusion: Neonatal exposure of BPA to rats impairs their fertility and has the potential to induce perturbations in SCJP. These perturbations may be one of the contributing factors that lead to impairments in spermatogenesis in the exposed animals and can be used as potential biomarkers to study BPA-induced effects on testes.

1. Introduction

Bisphenol A (BPA), an endocrine disrupter, has generated a great deal of concern on the part of regulatory agencies and scientists due to its high level of production and widespread use in consumer products. BPA is a monomer that is used in the manufacture of polycarbonate plastics, epoxy resins and a multitude of consumer products. It easily leaches from the inner lining of tin cans and microwave containers into food during heating, from dental sealant into saliva and into beverages from repeated usage or contact with any acidic/alkaline content in polycarbonate bottles (Brotons et al., 1995; Olea et al., 1996).

The detection of BPA in biological fluids like maternal plasma, fetal plasma, placental tissue, amniotic fluid and umbilical cord blood have indicated that it can easily transverse the placental barrier (Vandenberg et al., 2007; Tsutsumi, 2005). A number of regulatory agency and risk assessment groups around the world have been investigating on the adverse effects of BPA and have concluded unanimously that at current exposure BPA has no health effects (NTP, 2001, 2008; European union risk assessment report, 2003; EFSA, 2008; vom Saal et al., 2007). Systemic and two- and three-generation toxicity studies have provided evidence to certify BPA as a "safe chemical" (Ema et al., 2001; Tyl et al.,

* Corresponding author. Tel.: +91 22 24192022/+91 22 24192139; fax: +91 22 24139412.
E-mail address: vanageg@nirrh.res.in (G. Vanage).

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2002). However, a lot of controversy still exists with respect to low dose effects of BPA. Experimental evidences on the impact of BPA on reproduction have generated a new paradigm for its toxicity evaluation that differs in a number of ways from the traditional toxicology (vom Saal and Hughes, 2005; vom Saal et al., 2005).

The effects of BPA on the fertility of male rats have remained contradictory (Al-Hiyasat et al., 2002; Richter et al., 2007). Kato et al. (2006) failed to observe any effect of BPA on the reproductive parameters of adult male rats after neonatal exposure, whereas Toyama and Yuasa (2004) observed transitory effects on the spermatogenesis and fertility of male rats exposed neonatally to BPA. These discrepancies in observations have been mainly attributed to differences in the doses, mode of exposure, the timings of treatment and the species used (Richter et al., 2007). Thus it has become imperative to investigate and validate the effects of BPA on the fertility of male rats and to delineate the possible mechanisms.

Programming of the hypothalamus-pituitary-testicular axis occurs during the neonatal period. Earlier reports on the exposure of estrogens to neonatal rats have documented its adverse effects on reproductive functioning, during adulthood. This suggests that the neonatal period is one of the most crucial periods that may be determinant of fertility in adulthood (Goyal et al., 2003). Indeed recent reports have suggested that neonatal but not adult exposure to BPA has significant effects on spermatogenesis and spermiogenesis in male indicating that BPA has time specific effects on certain estrogen-sensitive stages of development (Toyama et al., 2004; Toyama and Yuasa, 2004). Hence the present study aimed at investigating the effects of BPA exposure during the critical "neonatal" period of development.

Sertoli cells and their junctions are known to play a pivotal role in the process of spermatogenesis. Inter-Sertoli cell junctions (i.e., adhesion (AJ), gap (GJ) and tight junctions (TJ)) are important for maintaining spermatogenesis. Connexins (Cx), are GJ proteins present in the testes. Amongst them, the most predominant is Cx43, which plays an essential role in spermatogenesis (Risley et al., 1992). Similar to the GJs, the Sertoli cell TJs (viz. Zona Occludens) are the primary constituents of the basal Ectoplasmic Specialization (ES) and create the blood testis barrier, which provides a specialized niche for germ cell development (Cheng and Mruk, 2002). The cadherin superfamily (viz. N-cadherin, E-cadherin) similarly significantly contributes to germ cell maturation and is one of the pivotal members of the AJs. Perturbations in these Sertoli cell junctional proteins (SCJPs) viz. Cx43 (GJ) and N-cadherin (AJ) have been widely associated with impairments in spermatogenesis in mice and rats (Batias et al., 1999; Sobarzo et al., 2006). These reports have implicated the significance of these SCJPs in spermatogenesis. Exposures to testicular toxicants have been reported to induce GJ and TJ disruptions in in-vitro studies (Gray and Beamand, 1984; Sinha et al., 1999). Exposure to Sertoli cell toxicants, such as phthalate, associated them with perturbations at the blood testis barrier found near the junctions (Richburg and Boekelheide, 1996). Thus alteration in the expression pattern of these SCJP may be potential markers for assessment of testicular toxicity. While growing evidences suggests BPA can act as a potential testicular toxicant that alters spermatogenesis, its role as a Sertoli cell toxicant has however largely remained obscure. In vitro studies have shown that the expression of SCJP such as Zona Occludens, N-Cadherin and Cx-43, are decreased in Sertoli cell lines after exposure to BPA (Fiorini et al., 2004). However, to the best of our knowledge, no information is available on the in vivo effects of neonatal exposure to BPA on these SCJPs.

In the present study we investigate the effect of neonatal exposure of BPA on fertility of male rats at adulthood and provide documentary evidence that BPA is a potential Sertoli cell toxicant.

2. Materials and methods

2.1. Chemicals and reagents

Bisphenol A (99.8% purity) and Diethylstilbestrol (DES, 99% purity) were purchased from Sigma Chemical., St. Louis, MO. Antibody to Testosterone and Estradiol were obtained from WHO and ICN, respectively, for performing radioimmunoassay. Standards for Luteinizing Hormone (LH; NIADDK-rat-LH-RP2) and Follicle Stimulating Hormone (FSH; NIADDK-rat-FSH-RP2) were obtained from NIH. Standards for Testosterone (T) and Estradiol (E) were obtained from Sigma Chemical Co., St. Louis, MO. Mouse monoclonal antibodies for tight junctional proteins (zona occludin-1 (ZO-1), adherens junctional proteins (N-cadherin) and gap junctional proteins (connexin-43 (Cx-43)) were purchased from Zymed (San Francisco, CA). Blocking solution and secondary antibodies were used from Immunocruz mouse staining kit (Santacruz Biotechnology Inc., Burlingame, CA).

2.2. Dose selection and preparation

The doses of BPA (100–1600 μg/kg bw) used in the present study were well below the published No Observed Adverse Effective Levels (NOAEL) dose, i.e., 5 mg/kg bw/day (NTP, 2001). BPA and DES were dissolved in a minimal amount of ethyl alcohol (99% pure) and then diluted in sesame oil to obtain the desired concentration of BPA dose range, i.e., 0.6, 1.2, 2.4, 5 and 10 μg/30 μl that corresponds to approximately 100, 200, 400, 800 and 1600 μg/kg bw of BPA, respectively. DES, which was previously reported to cause adverse effects in experimental animals at 10 μg/30 μl (corresponding approximately to 1600 μg/kg bw), was selected as a positive control in the present study (Goyal et al., 2003; Sharpe et al., 1998). Control groups received sesame oil with the same alcohol concentration. An additional control group that had received sesame oil without ethanol was also incorporated into the present study. Dose formulations were stored in amber colored bottles at 37 °C overnight and were subsequently kept at room temperature throughout the study. Solutions were mixed thoroughly before use.

2.3. Animal care and maintenance

Holtzman strain male and female rats (9 weeks of age, weighing ~250 g) that were randomly bred in our animal house were used in the present study. The animals were kept in polypropylene cages with autoclaved paddy husk for bedding and maintained at controlled temperature (23 ± 1 °C) and humidity (55 ± 5%), with a 14-h light/10-h dark cycle. Animals were fed a diet of soy-free, in-house-prepared rat pellets (consisting of crude protein, fiber and nitrogen free extract) and water (purified by UV and reverse osmosis) *ad libitum* throughout the study. The quality of food and water provided was routinely monitored by qualitative and quantitative proximal analysis. Ethical clearance for the use of animals in the study was obtained from the Institutional Animal Ethics Committee prior to the initiation of the study, and the experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), India.

2.4. Experimental design

The copulated female rats (12 rats/group) were allowed to deliver their offspring naturally. The day of delivery of the pup was considered to be post-natal day (PND) 0, and the litter size was adjusted to 4–5 male pups/litter. Neonatal male pups (32 pups/dose/group) were treated by daily subcutaneous injections during PNDs 1–5 with BPA (0.6 μg, 1.2 μg, 2.4 μg, 5 μg, 10 μg) in