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Genistein reduces the noxious effects of in utero bisphenol A exposure on the rat prostate gland at weaning and in adulthood

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ABSTRACT

Bisphenol A (BPA) is one hormonally active chemical with potential deleterious effects on reproductive organs, including breast and prostate. In contrast, genistein (GEN) is the major phytoestrogen of soy that presents potential protective effects against hormone-dependent cancers, including that of the prostate. Thus, pregnant Sprague–Dawley rats were treated with BPA at 25 or 250 μg/kg/day by gavage from gestational day (GD) 10–21 with or without dietary GEN at 250 mg/kg/chow (~5.5 mg/kg/day). Then, male offspring from different litters were euthanized on post-natal day (PND) 21 and 180. At PND21, BPA 25 exposure induced early prostatic changes while dietary GEN attenuated some deleterious actions this xenoestrogen on epithelial cell proliferation levels, androgen receptor expression and prostatic architecture in male offspring. At PND180, a significant increase in incidence of prostatic multifocal inflammation/reactive hyperplasia and atypical hyperplasia were observed in male offspring from dams that received BPA 25. On the other hand, maternal GEN feeding attenuated some the adverse effects of BPA 25 on prostate disease at late-in-life. This way, the present findings point to preventive action of dietary GEN on deleterious effects of gestational BPA exposure in both early and late prostate development in offspring F1.

1. Introduction

Bisphenol A (BPA) is a synthetic compound widely used to make polycarbonate plastic and epoxy resins present in thousands of household and consumer products, including coating materials for food cans and metal lids, in dental sealants and as finishing and coating materials for PVC pipes, food packaging materials, plastic bottles, baby bottles, and many other products for human daily use (Rubin, 2011). This chemical has been categorized as an endocrine disrupter (ED) owing to their ability to modulate the hormone-dependent organs, including prostate and breast (Rubin, 2011; Rochester, 2013).

Since BPA is one of the highest production volume chemicals in the worldwide commerce (Rubin, 2011; Vandenberg et al., 2007, 2010), a significant increase in exposure due to dermal absorption, inhalation, and ingestion from contaminated food and water will be expected (Geens et al., 2012). In the epidemiological study conducted by the National Health and Nutrition Examination Survey (NHANES) of American Center for Disease Control (CDC) from 2003 to 2004 was observed significant amounts of BPA in urine in about 93% of American adults, with highest levels being found in infants and children (Calafat et al., 2008).

Also, urinary BPA levels (low < 1.9; moderate 1.9–4.1; high > 4.1 ng/ml) were potentially associated with overall delayed menarche, especially for moderate levels of BPA exposure in a sample of 987 adolescent girls from NHANES 2003–2010 (McGuinn et al., 2015). Also, low levels (2–4 ng/ml) of unconjugated or free BPA have been detected in adult serum (Vandenberg et al., 2007, 2010). However, higher levels have been reported in amniotic fluid, fetal circulation, and neonates (Padmanabhan et al., 2008; Vandenberg et al., 2007, 2010), indicating a potential noxious for BPA to act as an ED in the different developing prostate periods.

Currently, a dose of 50 μg/kg/day is considered 'safe' for daily human consumption by the USEnvironmental Protection Agency (EPA) based on Lowest Observable Adverse Effect Level (LOAEL,

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50 mg/kg) divided by 1000-fold safety factors because a no observed adverse effect level (NOAEL) had not been clearly determined in rodent studies (NTP, 1982). However, some studies have shown that environmentally relevant doses of BPA (<50 μg/kg/day) during pregnancy can result in morphological and functional prostate changes and in increased risk for prostate cancer development in both adult male rats and mice (Ramos et al., 2001; Ho et al., 2006; Ogura et al., 2007; Prins et al., 2008; Brandt et al., 2014). In addition, some studies have demonstrated that BPA is easily transferred across the placenta, indicating a potential risk for rat fetuses (Takahashi and Oishi, 2000; Doerge et al., 2011).

Several epidemiological and experimental evidences points to the involvement of dietary compounds in the prevention of various human diseases, including prostate cancer development (Syed et al., 2007; Bommreddy et al., 2013). Numerous dietary phytochemicals have been observed to inhibit the initiation, promotion and progression phases of prostate carcinogenesis, including soy isoflavones (Lamartiniere et al., 2002; Ahmad et al., 2013). Genistein (GEN, 4,5,7-trihydroxyisoflavone) is main phytoestrogen found in soy beans and human intake of this isoflavone in both conjugated or unconjugated (aglycone) forms comes primarily from consumption of soy foods such as tofu, soy milk, sour flour, textured soy protein, tempeh, and miso (Xiao, 2008). Plasma concentrations of 50–800 ng/ml have been found for GEN in adults who consume modest amounts of soy foods Setchell and Cole, 2003). Thus, fetus can be actively exposed to GEN by mothers who consume soy products during pregnancy since a high correlation has been observed for isoflavone serum levels between maternal and umbilical cord blood samples, indicating that GEN can be easily transferred to the fetus by placenta (Nagata et al., 2006). This isoflavone is often categorized as a phytoestrogen because of its ability to bind to estrogen receptors (ERs) but display weak estrogenic activity when compared to the estrogen (Vitale et al., 2013).

GEN has demonstrated antitumor properties in different tumor cell lines and in rodent hormone-dependent organs such as the prostate and breast (Lamartiniere et al., 2002; Taylor et al., 2009; Mahmoud et al., 2014). Reduced prostate tumor development has been observed in transgenic and chemically-induced rodent models after dietary intake of genistein at human relevant concentration (Mahmoud et al., 2014). Also, maternal GEN exposure at the human intake dose level did not induce adverse effects on fetus development or in the reproductive organs in the rodent offspring F1 (Kang et al., 2002; Fielden et al., 2003; McClain et al., 2007).

Since that prenatal BPA exposure occur at environmentally relevant doses and can cause significant adverse effects at late in life (Vandenberg et al., 2007, 2010; Teeguarden and Hanson-Drury, 2013; Shelnutt et al.,. 2013), this study was established to investigate whether maternal GEN intake could to reverses the harmful effects of in utero BPA exposure on the early prostate morphogenesis and ultimately, the susceptibility to the development of prostatic lesions in adulthood. Additionally, this study aimed at evaluating the modifying effects of the interaction between these two estrogen-like compounds that do not have same properties when given alone, in part because of their different properties on the estrogen receptors (Yoon et al., 2014).

2. Materials and methods

2.1. Animals and experimental conditions

Animals were handled in accordance with the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation (COBEA), and the experiment was approved by the Committee of Ethics in Animal Use (CEUA) from School of Medicine, UNESP, Botucatu-SP, Brazil (Protocol number 1055/2013).

Male and female Sprague–Dawley (SD) rats were obtained from colonies maintained under specific pathogen-free conditions in the Multidisciplinary Center for Biological Investigation (CEMIB-UNICAMP, Campinas-SP, Brazil). All animals were housed in polypropylene cages with autoclaved white pine shavings as the bedding material maintained under controlled environmental conditions (temperature: 22 ± 2 °C; relative humidity: 55% ± 20%; 12/12-h light–dark cycle; and continuous air exhaust). All animals received free access a phytoestrogen free Nuvilab CR-1 commercial chow (Nuvital, PR, Brazil) and glass bottles containing free BPA drinking water. The polypropylene cages were cleaned manually and dried at room temperature to avoid exposure to high temperatures and BPA release (Howdeshell et al., 2003).

After a 2-week acclimation period, 8-week-old female SD rats were mated with 12-week-old male SD rats by placing 2 females in a cage with 1 male. Mating was realized during the dark period of the cycle and gestational day (GD) 0 was determined by the presence of sperm in the vaginal smears of female rats in estrus (sexually receptive) cycle. The mating process was repeated (do not exceed 5 times per female) when females SD did not show the presence of sperm in the vaginal smear. Because the phases of the estrus cycle present small and varying duration, the mating process occurred all day until the number of pregnant SD rats to complete the different experimental groups. Food and water consumption from the dams and the litters were weekly recorded.

2.2. Experimental design

Pregnant SD rats were orally treated by gavage with 25 or 250 μg/kg/day, respectively) body weight of BPA (CAS no. 80-05-7, Sigma–Aldrich Co®., USA) or dimethyl sulfoxide vehicle (DMSO; CAS no. 67-68-5, Sigma–Aldrich Co®., USA) from GD10 to GD21. BPA was dissolved in DMSO and solubilized in canola oil (1% final DMSO volume) and two solutions were prepared at final concentrations of 5 μg/mLor 50 μg/mL of BPA. The first solution (5 μg/mL) was used in dams exposed to BPA at 25 μg/kg body weight; and the other solution (50 μg/mL) was used in dams exposed to BPA at250 μg/kg body weight (Brandt et al., 2014). During GD10 to 21, dams received basal diet or basal diet containing GEN (99% purity and gently donated by DSM Nutritional Products – Basel, Switzerland) at concentration of 250 mg/kg chow and drinking water ad libitum. Five experimental groups (n = 10 dams/group) were as follows: G1, control group (vehicle plus basal chow); G2, BPA 25-treated group (25 μg/kg/day BPA plus basal chow); G3, BPA 250-treated group (250 μg/kg/day BPA plus basal chow), G4, BPA 25 plus GEN-treated group (25 μg/kg/day BPA + basal chow containing GEN at 250 mg/kg); G5, BPA 250 plus GEN-treated group (250 μg/kg/day BPA + basal chow containing GEN at 250 mg/kg). BPA 25 μg/kg/day was one-half of the daily tolerable dose of BPA 50 μg/kg/day established by US EPA while BPA250 μg/kg/day was >200-fold less than the lowest observable adverse effects level of 50 mg/kg/day (NTP, 1982).

GEN is easily transferred by placenta in pregnant female SD rats (Doerge et al., 2001) and the 250/mg/kg chow is a maximally tolerated dose in the diet that is not toxic to the prostate differentiation in male offspring from pregnant female SD treated with this isoflavone (Fritz et al., 2002a). Also, this maternal dietary level result in serum concentration of 726 nmol/L in 7-day-old offspring (Fritz et al., 1998), which approximated levels found in Japanese populations eating traditional soy-rich diet (Adlercreutz et al., 1993).

After parturition, the litter size was standardized to eight pups (the gender ratio was kept as close to 1:1 as possible) per dam in all the experimental groups. After weaning, at post-natal day (PND) 21 (n = 20 males/group; 2 males/litter) and PND180 (n = 10 males/