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Interactive effects of nonylphenol and bisphenol A exposure with oxidative stress on fetal reproductive indices

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ABSTRACT

Nonylphenol (NP) and/or bisphenol A (BPA) may have reproductive effects. Although the mechanisms of action remain unclear, steroid hormones biosynthesis, hypothalamus pituitary adrenal axis activity, oxidative stress, and crosstalk interaction of NP and BPA mixture and its pathways may play a contributory role. This cross-sectional study examined whether the interactive effects of NP/BPA and oxidative stress biomarkers played a role in reproductive indices (penis length and anogenital distance (AGD)) in 244 mother-fetus pairs. Four biomarkers of oxidative stress, (8-hydroxy-2′-deoxyguanosine (8-OHdG), 8-nitroguanine (8-NO_2Gua), 8-iso-prostaglandin $F_{2\alpha}$ (8-isoP$F_{2\alpha}$), and 4-hydroxy-2-nonenal-mercapturic acid (HNE-MA)) were simultaneously analyzed using the high-performance liquid chromatography-electrospray ionization tandem mass spectrometry method. No significant associations were found between reproductive indices and NP/BPA or oxidative stress biomarkers. Maternal exposure to a mixture of NP and BPA may enhance 8-OHdG. Interactive effects were found in the high 8-isoP$F_{2\alpha}$ group, and prenatal NP exposure was inversely associated with penis length ($\beta = -3.68$ mm; $p = 0.01$). Similar results were noted among boys who were born to mothers in the high 8-isoP$F_{2\alpha}$ group, in which BPA was inversely associated with penis length ($\beta = -4.43$ mm; $p = 0.005$). Our findings suggest important implications for prenatal exposure to oxidative stress, as evidenced by the 8-isoPF2α level. Thus, NP and BPA may interact to shape fetal reproductive tract development, particularly in boys. The interactive effects of NP/BPA, oxidative stress, and reproductive indices should be considered.

1. Introduction

Bisphenol A (BPA) and nonylphenol (NP) are known endocrine-disrupting compounds (EDCs) that can mimic natural hormones. These manmade alkylphenolic chemicals are widely produced worldwide. NP is employed in formulation of detergents, surfactants, and cosmetics, whereas BPA is utilized for the production of epoxy resins and polycarbonate plastics in multiple daily products (CDC, 2014; Ying et al., 2002). It has been reported that BPA and NP are common contaminants in Taiwan (Huang et al., 2014, 2017a). Widespread exposure of humans to NP/BPA is evident, because more than 90% of the general population has measurable BPA levels in their urine (Vandenberg et al., 2010) and blood (Chou et al., 2011). Significantly higher NP levels have been detected in the Taiwanese population than in USA (Calafat et al., 2005) and Japanese (Kawaguchi et al., 2005) populations.

Experimental studies have reported that both BPA and NP affect male fertility and reproduction and reduce the anogenital distance (AGD) (Al-Hiyasat et al., 2002; Christiansen et al., 2013; Jie et al., 2010). The AGD, which is the distance between the anus and the genitals, is a sensitive marker of androgen and anti-androgen exposure during the critical masculinization programming window (MPW) (Thankamony et al., 2016). The AGD, was recommended as one of the endpoints for examination of the reproductive toxicity of EDCs by an international workshop held in Canada (Arbuckle et al., 2008). In humans, two studies have reported inconsistent findings in relation to BPA or NP exposure and the AGD; one study reported a significant negative

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association with BPA (Miao et al., 2011a), but a recent study did not identify an association between BPA and NP (Liu et al., 2016). However, these findings were based on a single chemical exposure. Recently, we have elucidated the null effects of concurrent exposure to an EDC mixture (including NP, BPA, mono-methyl phthalate, Σphthalate, diethylphosphate, and Σdiethylphosphate) on birth outcomes, possibly due to the relatively small sample size (Huang et al., 2017a). Little is known about the impact of simultaneous exposure to BPA and NP on reproductive effects.

The structures of NP and BPA are similar to the structure of estrogen, and these chemicals exhibit both estrogenic and anti-androgenic effects (Xu et al., 2005). Although the modes of action of NP/BPA-induced effects on AGD have not been elucidated, NP and BPA may interrupt androgen receptor (AR)-binding activity, disrupt androgen action during the MPW and induce reactive oxygen species (ROS) signaling (Chitra et al., 2003; Lee et al., 2003; Liu et al., 2014). Concurrent exposure to EDCs with crosstalk and common mechanisms occurs in the environment and may lead to dynamic interactions and effects that can be additive, synergistic or antagonistic (Sharma et al., 2017). Studies have shown that NP inhibits testosterone release in rat Leydig cells (Wu et al., 2010), and a similar finding has been reported for BPA in human H295R cells (Zhang et al., 2011). Simultaneous exposure to NP and BPA may cause additive or synergistic reproductive effects.

Oxidative stress, which has been defined as an imbalance between reactive oxygen/nitrogen species (ROS/RNS) and antioxidants in the body, leads to spermatozoa damage and eventually male infertility (Agarwal et al., 2008; Aly et al., 2012). ROS/RNS may react with DNA or lipids, resulting in oxidative and nitrative damage to DNA and lipid peroxidation (Halliwell, 1991). Studies have reported that NP/BPA induce oxidative stress in testicular Sertoli cells and rat epididymal sperm and that co-exposure to NP and BPA results in an additive effect on antioxidant inhibition in zebrafish embryos (Aly et al., 2012; Chitra et al., 2003; Gong and Han, 2006; Wu, 2011). Monitoring prenatal oxidative stress is vital for elucidating the connection between adverse effects and oxidative stress in mother-fetus pairs. Recently, we reported associations among maternal NP level and four oxidative stress biomarkers, including products of oxidative DNA damage (8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-nitroguanine (8-NO_2Gua)) and lipid peroxidation (8-iso-prostaglandin $F_{2\alpha}$ (8-iso$PF_{2\alpha}$) and 4-hydroxy-2-nonenal-mercapturic acid (HNE-MA)). Additionally, we reported that the 8-OHdG concentration was inversely associated with gestational age (Wang et al., 2015a, b). However, evidence of the effects of NP and BPA co-exposure on oxidative stress and the effects of oxidative stress on reproductive organs development in humans is rare and needed. Therefore, we performed this study to explore (a) exposure to NP, BPA and their mixture in relation to reproductive indices and oxidative stress biomarkers and (b) to further characterize the interaction of NP/BPA and oxidative stress on reproductive indices.

2. Materials and methods

2.1. Subjects

The study subjects were enrolled in Taipei City Hospital (TCH) (Huang et al., 2017b), and the protocol was approved by the TCH institutional review board. Briefly, mother-baby pairs were recruited as follows: women who were cancer free, an age of 18–45 years, at gestational weeks 27–38; their fetuses were healthy and carried to term (> 37 weeks pregnant). We excluded women who smoked and drank during pregnancy or had occupational exposure to BPA/NP. A total of 244 mother-baby pairs completed the follow-up between 2014 and 2016. The woman provided single spot urine samples at TCH between weeks 27 and 38 of gestation and completed a structured questionnaire. The questionnaire collected demographic characteristics (age, education, and prepregnancy body mass index (BMI)), personal lifestyle habits, dietary data, and disease history. The urine samples were stored at −20 °C prior to analysis. The containers used for sampling and storage were NP- and BPA-contamination free.

2.2. Physical examination of newborns

At delivery, the birth weight and height, gestational age, penis length, and AGD of the newborns were measured and recorded by a single pediatrician. For boys, the AGD was measured from the center of the anus to the junction of the perineal skin with the rugated skin of the scrotum; for girls, the AGD was measured from the center of the anus to the posterior convergence of the fourchette (Salazar-Martinez et al., 2004). The AGD was measured in triplicate to obtain the average level. Additionally, we normalized and calculated the anogenital index (AGI) based on the individual birth weight (-W) and length (-L). The AGI-W was the AGD ÷ birth weight, and the AGI-L was the AGD ÷ birth length.

2.3. Laboratory analyses

2.3.1. Measurement of creatinine

Creatinine level was determined using the Jaffe reaction (Jaffe, 1886) and analyzed by a spectrophotometer. Twenty-seven urine samples were excluded from the statistical analysis because creatinine levels were outside of the reference range (< 0.3 g/L or > 3.0 g/L). The markers of oxidative stress, BPA, and NP were normalized to creatinine and expressed as μg/g creatinine.

2.3.2. Urine analyses for NP and BPA

No plastic products were employed during sample pretreatment. Sample preparation and analyses of NP and BPA followed previously published procedures (Wang et al., 2015b) with minor modifications (Lai, 2016). Briefly, 2 mL of each urine sample was spiked with 50 μL of 2000 ng/mL $^{13}C_{12}$-BPA as an internal standard (IS). The isotope-dilution sample was adjusted to pH 5.5, deconjugated using β-glucuronidase/arylsulfatase (Sigma-Aldrich, USA), incubated at 37 °C for 15 h, and then acidified to pH 3 using 1 M hydrochloric acid. Next, the samples were extracted using Varian PH solid-phase extraction (SPE) and were eluted with 1 mL of methanol. The method used to determine the NP levels was previously described (Wang et al., 2015b) and used high-performance liquid chromatography (HPLC) coupled with fluorescence detection (Hitachi, Tokyo). BPA was analyzed using an Acquity ultra-performance liquid chromatography system (Waters, MA, USA) coupled to a time-of-flight mass spectrometer (Waters, MA, USA). BPA was separated on a BEH C18 column (1.7 μm, 2.1 × 10 mm, Waters, USA) with a flow rate of 0.35 mL/min. Chromatography was performed using the following gradient program: an initial mobile phase composed of 20% methanol with 0.1% ammonia and 80% water with 0.1% ammonia for 0.5 min, followed by an increase to 99% methanol with 0.1% ammonia for 1.5 min, a return to the initial condition for 1 min and equilibration for 3 min. The MS/MS was operated for BPA quantification by monitoring ion mass transitions as follows: m/z 227.12→133.07 for BPA and m/z 239.18→139.09 for $^{13}C_{12}$-BPA. A calibration curve was constructed with pooled urine samples spiked to a level range of 0.5–100 ng/mL. The linear calibration curve was obtained by plotting the quotient of the peak areas of BPA and $^{13}C_{12}$-BPA versus the standard levels. The limit of detection (LOD) was determined by analyzing the lowest concentration of the calibration curve seven times. The LOD was calculated as three times the standard deviation divided by the mean value of the peak area multiplied by the lowest detectable concentration. To validate the method performance, calibration standards, blank and spiked samples of human pooled urine were analyzed in each batch. The average recoveries were 77–105% (6–235 ng/mL) for NP and 93–100% (20–100 ng/mL) for BPA, respectively. The LODs in urine were 0.20 ng/mL for NP and 0.16 ng/mL for BPA, and the calibration curves displayed excellent linearity with regression coefficients (R^2) greater than 0.995. The intra- and inter-day variations were 13% and