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In utero and lactational exposure to di(2-ethylhexyl) phthalate increased the susceptibility of prostate carcinogenesis in male offspring

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ABSTRACT

di(2-ethylhexyl) phthalate (DEHP) is one of the most commonly-used plasticizers and can exert estrogen-like effects and anti-androgen-like effects after entering the body. The critical windows for DEHP exposure are in utero and during lactation. There's substantial evidence that hormonally active environmental estrogen perturbations in early life are associated with prostate carcinogenesis susceptibility later in life. In order to explore the effects of *in utero and lactational* exposure to DEHP on the male offspring's susceptibility to prostate carcinogenesis, we established a rat model of developmental estrogenization: pregnant rats in three treatment groups were treated with DEHP at 0.01, 0.1 and 1 mg/kg BW/day from GD7 to PND21, and the male pups in positive group were treated with EB at 25ug/pup on PND 1, 3, 5. In order to induce the prostate carcinogenesis, half of the male offspring were given implants packed with estradiol and testosterone, and the other half were given empty tubes on PND90. The prostate weight, concentration of PSA in serum and histopathological changes were measured in male offspring on PND196. Data was analyzed by one-way analysis of variance (ANOVA) and x^2 using SPSS Statistics software. Results showed that in utero treatment of DEHP decreased the prostate weight, prostate/body weight ratio and increased PSA concentrations of male pups in a dose-dependent manner. Compared with non-T + E treatment groups, T + E treatment increased the prostate weight and ratio of prostate/body weight, as well as the concentration of PSA. The results of prostate carcinogenesis parameters including PIN scores/frequency and Gleason scores/frequency were consistent with PSA, which meant that *in utero and lactational* exposure of DEHP was a risk factor of prostate carcinogenesis, and the increased estrogen/testosterone (E/T) ratio in adult might exert synergistic effects in the process of prostate carcinogenesis formation.

1. Introduction

There are increasing worldwide concerns about prostate carcinogenesis, as it is the second leading cause of cancer death in men. Prostate cancer remains a significant disease burden in developed countries: account for 94,000 deaths in Europe and 85,425 in the United states in 2012 [1]. In China, studies evaluating prostate carcinogenesis have previously suggested that the prevalence of prostate carcinogenesis is 3%–20% [2].However, a recent study of 340 Chinese cystoprostatectomy specimens revealed that 28% of specimens had incidental prostate carcinogenesis identified, suggesting that the true prevalence of prostate carcinogenesis in Chinese men is significantly higher than previously reported and perhaps comparable to that of western population [3,4].

In human, prostate morphogenesis occurs from the second and third trimester to the time of birth; in rodent animals, prostate morphogenesis take place from late fetal life and the time of birth to the first 15 days of life [5]. The proper development of prostate is largely dependent on the constant supply and binding of circulating testosterone or its more potent metabolite, 5-dihydrotestosterone (DHT) to androgen receptors in the urogenital sinus (USG)

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mesenchyme and further activation of USG epithelium by mesenchyme. And the physiological shift of hormonal milieu is widely believed to be related to high incidence of prostate carcinogenesis in the older men. Although the prostate carcinogenesis is mainly occurs in older men, the Prostatic Intraepithelial Neoplasia (PIN) or high-grade Prostatic Intraepithelial Neoplasia(HPIN)have been identified in autopsies of 20–30 year old men [6],suggesting that prostate carcinogenesis may begin much earlier.

It has been proposed that excessive estrogenization during prostatic development may contribute to the high incidence of PIN and prostatic carcinoma in the aging male population [7]. For example, Palmer [8] et al. found that maternal exposure to diethylstilbestrol (DES) during pregnancy resulted in more extensive prostatic squamous metaplasia in male offspring than observed with maternal estradiol alone. Although the DES had been discontinued for pregnant women in the early 1970s, the recent studies has shown that certain environmental chemicals with potent estrogenic activities have led to a renewed interest in the effects of exogenous estrogens during prostatic development [9]. Developmental exposure to bisphenol A, one kind of estrogenic chemicals, has been found to increase the susceptibility to prostate carcinogenesis in animal studies, which indicate the hormonally active xenoestrogens' effects on reproductive and developmental processes of the organism and its descendants (mainly affect male offspring) cannot be neglected [10].

DEHP is a typical environmental chemical with both estrogen-like effects and anti-androgen-like effects [11], which is widely used in a range of consumer products including cosmetics, plastics, carpets, building materials, toys and medical and cleaning products. Previous studies have documented that environmental exposure to DEHP have adverse effects on reproductive system including the reductions in the semen count and quality [12], decreased testosterone in serum [13], disturbed hypothalamic–pituitary–testis axis in male rats [14], and reduced androgen receptor protein level in LNCaP cells[15], while the carcinogenic effects of DEHP were found in different target tissues, notably in liver and testis [16]. However, researches about the effects of exposure to DEHP on the prostate are few. Considering the anti-androgenic and estrogenic activities of DEHP and its sensitive exposure window, we hypothesize whether *in utero and lactational* exposure of DEHP has any effects on the susceptibility to prostate carcinogenesis in male offspring. Based on animal models from Shuk Mei's study [10], we tend to explore DEHP's effect on prostate cancer's susceptibility and what role does the increased estrogen/testosterone (E/T) ratio play in the process of prostate carcinogenesis formation.

2. Materials and methods

2.1. Chemicals and materials

DEHP (Chemsrvice, America), 17-estradiol 3-benzoate(EB) (Chemsrvice, America), corn oil (Chemsrvice, America), silastic capsules(inside diameter 1.5 mm, outer diameter 2.0 mm; Dow Corning, Midland, MI), estradiol (Sigma-Aldrich Chemical Co, America), testosterone (Sigma-Aldrich Chemical Co, America), Rat PSA Elisa Kit (TSZ BIO, America), 70% ethanol, Microplate Reader (Tecan Infinite 200 PRO, Sweden), Microscopy (Olympus, America).

2.2. Animal housing

All animal procedures were reviewed and approved by Shanghai Institute of Planned Parenthood Research. Sprague-Dawley rats were purchased from Shanghai SIPPR-BK Experimental Animal Co. Ltd. The adult Sprague-Dawley rats were brought on 60 days, and the weights of them were between 250 g to 350 g. They were housed in SPF (special pathogen free) laboratory of Shanghai Institute of Planned Parenthood Research, under a controlled12-liight: 12-dark cycle.

After feeding a week to adapt to the new environment, the male and female adult rats were put together at a cage with the ratio of 1:2 at night. We checked whether these female rats were pregnant by vaginal smears, and the day of finding sperm in the vagina was

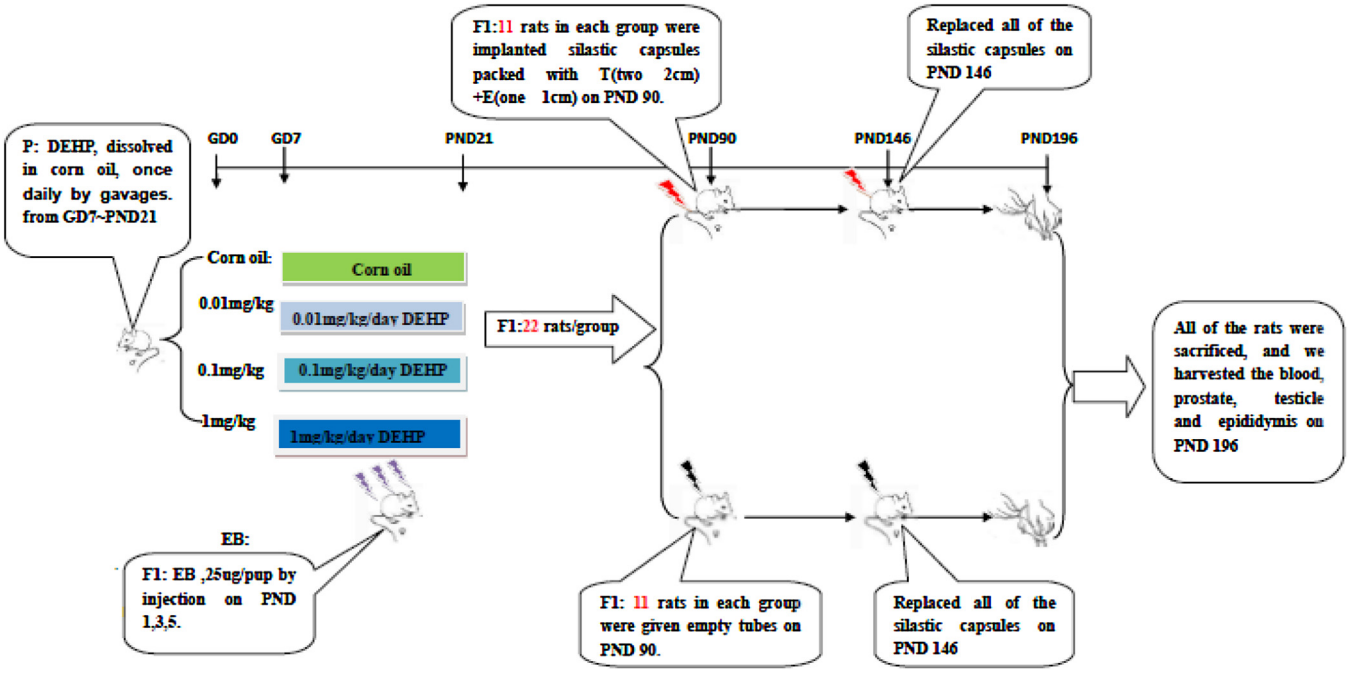

Fig. 1. Schematic representation of F1 rat animal model for in utero and lactational exposure to DEHP followed by second exposure to T + E or empty implants. The purple arrowhead means being treated with EB at 25 ug/pup on postnatal days 1, 3 and 5 by injections in the nape of the neck. The red and black arrowheads indicate times for implanting silastic capsules with T + E and none respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)