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Review

Early programing of uterine tissue by bisphenol A: Critical evaluation of evidence from animal exposure studies

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ABSTRACT

Exposure to Bisphenol A (BPA) during the critical window of uterine development has been proposed to program the uterus for increased disease susceptibility based on well-documented effects of the potent xenoestrogen diethylstilbestrol. To investigate this proposal, we reviewed 37 studies of prenatal and/or perinatal BPA exposure in animal models and evaluated evidence for: molecular signatures of early BPA exposure; the development of adverse uterine health effects; and epigenetic changes linked to long-term dysregulation of uterine gene expression and health effects. We found substantial evidence for adult uterine effects of early BPA exposure. In contrast, experimental support for epigenetic actions of early BPA exposure is very limited, and largely consists of effects on *Hoxa* gene DNA methylation. Critical knowledge gaps were identified, including the need to fully characterize short-term and long-term uterine gene responses, interactions with estrogens and other endogenous hormones, and any long-lasting epigenetic signatures that impact adult disease.

Contents

Abbreviations: BPA, bisphenol A; ER, estrogen receptor.

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1. Introduction

Exogenous exposure to natural hormones and hormone-mimetic chemicals during early life can induce permanent changes in development, and is proposed to increase disease risk during adulthood [1]. This hypothesized developmental origin of adult disease has been widely discussed in relation to the potential long-term effects of exposure to xenoestrogens [2,3] and derives strong support from the reproductive abnormalities seen in female offspring of women exposed to diethylstilbestrol (DES) during pregnancy [4]. In utero exposure to DES, a potent estrogenic chemical, was first associated with development of clear cell adenocarcinoma of the cervix in young women [5]. Other abnormalities emerged as DES daughters aged, including decreased fertility, increased rates of ectopic pregnancy, and early menopause [6]. The widespread and persistent exposure of humans and other mammals to other environmental estrogens, including the plasticizer bisphenol A (BPA), is a major public health concern [7,8].

BPA was long considered a weak estrogen, as its binding affinity for the estrogen receptors, ERα and ERβ, was estimated to be 1000–10,000-fold lower than that of 17β-estradiol [9,10]—in contrast to DES [11]. However, recent studies show that BPA can promote estrogen-like activities with a potency similar to or greater than that of 17β-estradiol, which may reflect alternative estrogenic mechanisms of BPA action [12], including rapid responses via non-classical estrogen signaling pathways [13,14] and differences in co-activator recruitment between BPA and 17β-estradiol [15]. Thus, emerging evidence of the estrogenic activity of BPA at low doses and its high affinity for uterine tissue [16], together with the fact that the developmental health effects of DES in humans are seen over a wide range of exposure doses [17], indicate a clear need to rigorously examine the extent to which BPA shares some of the well documented adverse health effects of DES, including reproductive toxicity [18].

BPA is widely used in the manufacture of polycarbonate plastics and epoxy resins, leading to significant exposure under normal conditions of use via food and beverage storage containers, impact-resistant baby bottles, dental-sealants and composites, and many other materials [19]. Substantial levels of BPA are readily measured in human tissue samples, including serum and urine from children, maternal and fetal plasma, amniotic fluid, and breast milk of nursing mothers [20–22]. BPA acts as a selective ER modulator, but can also bind to other hormone receptors and thereby impact multiple endocrine-regulated pathways [23–26]. BPA crosses the placental barrier [27] and has been linked to adverse human reproductive effects, including recurrent miscarriage [28,29]. In animal models, perinatal exposure to low, environmentally relevant doses of BPA induces developmental defects in brain function and behavior, the male reproductive system, and the mammary gland [30–32], where an increased predisposition to cancer has been observed [33]. BPA exposure is associated with several reproductive toxicities, as seen in animal models and/or in women. Thus, BPA affects meiosis in ovaries, accelerates follicle transition, reduces oocyte quality in animal models and in women undergoing in vitro fertilization, impairs uterine endometrial proliferation, decreases uterine receptivity, and increases implantation failure [18,34]. Given the prevalence of BPA exposure, as well as the long term deleterious effects of early DES exposure in the female reproductive tract, there is much interest in understanding the impact of early exposure to BPA on uterine tissue and any changes in developmental programing that may lead to adult-onset disease.

BPA and certain other environmental chemicals can disrupt the programing of cells and tissues by epigenetic mechanisms that induce long-term changes in chromatin structure and gene expression [31]. BPA-induced programing may involve several interrelated epigenetic mechanisms, including changes in DNA CpG methylation, alterations in histone modifications, and dysregulation of noncoding RNA expression [35]. Methylation of genomic DNA and/or covalent modification of the core histones that package DNA into nucleosomes, e.g., via histone methylation, acetylation, ubiquitylation, and sumoylation, may alter gene expression by changing chromatin packaging density and the accessibility of DNA for transcription factor binding. Short noncoding RNAs can silence genes by mRNA degradation, translation arrest, and miRNA-dependent chromatin remodeling, while long, intergenic noncoding RNAs (lincRNAs) can serve as molecular scaffolds that direct histone modifying enzymes to specific genomic loci [35–37].

There is increasing evidence in non-uterine tissues that BPA can introduce epigenetic changes involving one or more of the above mechanisms, with DNA methylation being the most frequently analyzed epigenetic outcome [31]. These studies cover multiple species, tissue/cell types, exposure paradigms and analyzed outcomes. However, the mosaic of reported results does not provide a clear and consistent mechanistic understanding of the epigenetic effects of BPA exposure. Examples of findings include increased DNA methylation of genes associated with tumor development in human mammary epithelial cells exposed to BPA [38] and the induction of several thousand differentially methylated genomic regions (DMRs) in postnatal day (PND) 21 rat mammary tissue following prenatal BPA exposure [39]. Further, BPA increased histone methyltransferase EZH2 and its repressive histone-H3 lysine-27 trimethylation marks, in MCF-7 breast cancer cells and in mammary tissue of mice exposed to BPA in utero [40]. In other studies, BPA exposure of rats during gestation and lactation altered glucose and insulin tolerance in F2 offspring in association with increased DNA methylation of the *Gck* gene in F2 liver, but with a decreased global level of DNA methylation in F1 sperm [41]. In testis of adult mice exposed to BPA neonatally, DNA methyltransferases *Dnmt3a* and *Dnmt3b* were up regulated and the promoters of *Esr1* and *Esr2* (which encode estrogen receptors ERα and ERβ) became hypermethylated [42]. In utero exposure to BPA induced dose-dependent changes in expression of *Dnmt1* and *Dnmt3a*, as well as *Esr1* and *Esr2*, in mouse brain, in a manner that is sex-dependent and region-specific [43]. *Esr1* gene DNA methylation was significantly increased in male prefrontal cortex and was decreased in the hypothalamus of females in association with disruption of sexually dimorphic social and anxiety-related behavior [43]. Further, the methylation status of *Not1* loci in mouse forebrain was altered, with some gene regions showing increased methylation and others showing decreased methylation following in utero BPA exposure [44]. CpG methylation was decreased in an intracisternal A particle retrotransposon (IAP) upstream of the *Agouti* gene [45]. BPA exposure in utero also disrupts genomic imprinting, leading to decreased methylation of DMRs and altered gene expression in mouse placenta [46]. Human exposure to BPA has been associated with decreased DNA methylation: BPA-exposed