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Estrogens in the wrong place at the wrong time: Fetal BPA exposure and mammary cancer

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ABSTRACT

Iatrogenic gestational exposure to diethylstilbestrol (DES) induced alterations of the genital tract and predisposed individuals to develop clear cell carcinoma of the vagina as well as breast cancer later in life. Gestational exposure of rodents to a related compound, the xenoestrogen bisphenol-A (BPA) increases the propensity to develop mammary cancer during adulthood, long after cessation of exposure. Exposure to BPA during gestation induces morphological alterations in both the stroma and the epithelium of the fetal mammary gland at 18 days of age. We postulate that the primary target of BPA is the fetal stroma, the only mammary tissue expressing estrogen receptors during fetal life. BPA would then alter the reciprocal stroma-epithelial interactions that mediate mammogenesis. In addition to this direct effect on the mammary gland, BPA is postulated to affect the hypothalamus and thus in turn affect the regulation of mammotropic hormones at puberty and beyond.

1. Introduction

Over the past four decades, strong evidence indicates that fetal exposure to estrogens causes perturbations in mammary gland development leading to mammary cancer manifested during adulthood [1–4]. Beside the effects reported from exposure to endogenous and pharmacological estrogens (diethylstilbisterol), a new source of concern is fetal exposure to endocrine disrupting chemicals (EDCs), including estrogen mimics (xenoestrogens) [5–7]. An EDC is an exogenous chemical, or a mixture of chemicals, that interferes with any aspect of hormone action [8]. Among these EDCs, BPA has gained much attention because of its ubiquitous presence in the environment [9–12]. The increased incidence of breast, uterine and testicular cancers observed in European populations in the past 50 years has been postulated to be due to EDC exposure during periods of increased vulnerability, such as fetal development and peri-pubertal stages [13–17]. This hypothesis was tested by examining the effect of environmentally relevant doses of BPA during fetal life of experimental animals. Perinatal BPA exposure to mice and monkeys resulted in alterations in both the stroma and the epithelium of the developing mammary gland [18–20]. Moreover, intraductal hyperplasias, ductal carcinoma *in situ* (DCIS) and palpable tumors in murine species were documented long after the end of exposure [21–26]. What remains to be determined is whether BPA acts exclusively by directly affecting epithelial-stromal interactions in the developing mammary gland or, in addition, by interfering with the hypothalamus-pituitary-ovarian axis (HPOA). In this review, we discuss the effects of fetal exposure to environmentally relevant levels of BPA on fetal mammary gland development and examine the hypothesis that BPA, by virtue of its estrogenicity, increases the risk of developing breast cancer in adulthood by exerting its deleterious effects through the stroma of the developing mammary gland.

2. Fetal exposure to endogenous and pharmacological estrogens

Epidemiological studies suggest that changes in the endocrine-fetal milieu predispose women to diseases that are manifested during adulthood [4,27–29]. For instance, non-identical twin birth is considered an indicator of high exposure to estrogen, while pre-eclampsia is regarded as an indicator of low exposure to estrogen.

Abbreviations: EDC, endocrine disrupting chemicals; BPA, bisphenol-A; DES, diethylstilbisterol; HPOA, hypothalamus-pituitary-ovarian axis; DCIS, ductal carcinoma *in situ*; GPR30, G-protein coupled receptor 30; ERR, estrogen related receptor; ECM, extracellular matrix; NMU, nitrosomethyl urea; TOFT, tissue organization field theory; PND, postnatal day; GD, gestational day; AVPV, anteroventral periventricular nucleus; EPA, environmental protection agency; DMBA, dimethylbenzanthracene.

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Dizygotic birth correlates with increased risk of breast cancer in the offspring while pre-eclampsia correlates with a lower risk [29–31].

Fetal exposure to pharmacological doses of estrogens increases breast cancer risk at the age of prevalence [28,30,31]. Compelling evidence favoring this claim was gathered from the daughters of mothers who were treated with massive doses of DES between 1948 and 1971 in order to prevent spontaneous abortion [32–35]. The "DES daughters", as these women are now commonly referred to, have been diagnosed with a variety of anatomo-physiological abnormalities in the uterus, oviduct and cervix as well as with clear cell adenocarcinoma of the vagina. Recently published data suggest that the DES daughters aged 40 years and older have an increased breast cancer incidence [1,36].

3. Mode of action of xenoestrogens during fetal life

Endogenous estrogens regulate multiple aspects of reproduction and development in males and females. Estrogens bring about their action by binding to nuclear receptors, estrogen receptor alpha [37] and beta [38] as well as the membrane-bound receptor, GPR30 [39]. In females, estrogens regulate the morphology and physiology of the reproductive tract at all stages of life, including puberty, pregnancy and menopause [40]. In males the generation of null mutants of the estrogen receptor (ER) alpha gene revealed that these mice were infertile. Also in males, it was shown that germ cells do not require ER alpha for development and function, but that somatic cells of the reproductive tract require ER alpha to produce sperm that are capable of fertilization [41]. During adulthood, endogenous estrogens mediate biological events in the reproductive tract that, for the most part, are reversible. However, fetal development of the female genital tract is thought not to require estrogens, and excess estrogens are known to produce irreversible alterations, such as those observed in DES daughters.

Xenoestrogens bind to estrogen receptors alpha and beta, both when residing in the nucleus and when attached to the plasma membrane. Additionally, xenoestrogens are known to bind to the membrane receptors GPR30 [42–44] and the orphan receptor estrogen related receptor gamma [45]. Low-dose effects of xenoestrogens may be due to their acting through membrane receptors and to additive action with endogenous estrogens [46].

4. BPA as a xenoestrogens

BPA is commonly used in the manufacturing of plastics and is present in polycarbonate products and epoxy resins. BPA exposure occurs when it leaches into containers holding food, beverages, water and milk, which are later consumed. Additionally, BPA is released from water pipes, dental materials, protective coatings, adhesives, protective window glazing, compact discs, thermal paper and paper coatings [9,47]. Pervasive use of BPA increases the risk of exposure to both the developing fetus indirectly through maternal exposure, and the neonate directly through ingestion of infant formula or maternal milk [11,48].

BPA is present in the urine of 95% of a representative sample of a non-institutionalized U.S. population over 6 years of age [49], including pregnant women [50]. BPA has also been detected in maternal and fetal serum and placental tissue of newborn humans. The range of BPA concentrations in fetal serum ranged from 0.2 to 9.2 ng/ml, indicating that the developing human fetus and neonate are readily exposed to this chemical [11]. To date, BPA is the best-studied xenoestrogen for which effects of exposure have been reported at various time points ranging from fetal to postnatal development.

5. Role of the environment during development

The influence of the environment on phenotype determination has been known since the 1880s from observations in wildlife species. For instance, August Weismann documented that the spring and summer morphs of a butterfly species could be regulated by temperature. However, the gene-centric view of development that dominated 20th century research overlooked these phenomena that revealed phenotypic plasticity [51]. The theory proposing that development is the execution of a genetic program gained prevalence from studies in animal models including Drosophila and rodents generated under strictly regulated laboratory conditions. Under these artificial conditions, animals reproduce all year long under light, temperature and strict diet regulations, avoiding external environmental influences. As a consequence, explanations in embryology have been narrowed down to genetics and molecular pathways. However, the phenomenon called polyphenism – one genotype, many phenotypes – contradicts the theory that development is the execution of a genetic program [51]. Lately, the role of the environment in phenotype determination is regaining central stage due to its relevance to disease. For example, epidemiological studies showed that malnutrition during fetal development increased the risk to coronary and metabolic diseases during adulthood. Thus the old phenomenon of developmental plasticity is coming back under a medical disguise, namely, the "fetal origins of adult disease" hypothesis [52].

6. Stromal-epithelial interactions in fetal mammary gland development

Besides being influenced by the external environment, embryonic development is mediated by interactions at various levels of biological organization. Epithelial cells interact with neighboring epithelial cells as well as with cells in the surrounding stroma and with the extracellular matrix (ECM). These interactions take place along several spatial and temporal scales that define the shape of the organism [53]. The organism imposes constraints at the local and global level *via* biophysical and biochemical interactions through cell proliferation, cell motility and cell adhesion. Tissue development in appendages like the mammary gland, tooth, feather and hair occur as a result of constant reciprocal interactions between epithelial cells and the surrounding ECM [54,55]. Pioneering work by Kratochwil showed that stromal-epithelial interactions are crucial for mammary gland morphogenesis [56]. Using an explant model and tissue recombination techniques, he separated embryonic day (E) 12–16 mammary epithelium from the mesenchyme, and showed that the epithelium develops only after recombination with its mesenchyme. In addition, he showed that recombination with the salivary mesenchyme induces the originally mammary epithelium to display a dichotomous branching pattern typical of the salivary gland. Later, Sakakura et al. confirmed these findings *in vivo*, by recombining E16 mammary epithelium with E14 salivary mesenchyme under the kidney capsule [57]. These results clearly pointed out the inductive role of the stroma in mammary and salivary gland morphogenesis.

7. Breast carcinogenesis: a consequence of altered tissue interactions?

Cell-based theories of carcinogenesis, such as the somatic mutation theory (SMT), consider cancer as a disease of cell proliferation and postulate that it occurs as a result of DNA mutations in genes that control cell proliferation [58]. Alternatively, the tissue organization field theory (TOFT) postulates that cancer, like morphogenesis is a matter of tissue organization, and it proposes cancer