Changes in Gene Expression Profile in the Rat Mammary Gland after Neonatal and Prepubertal Exposure to the Xenoestrogen Bisphenol A (BPA).

Raquel Moral[1], Gabriela A. Balogh[1], Daniel A. Mailo[1], Irma H. Russo[1], Coral A. Lamartiniere[2], and Jose Russo[1].
[1]Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, PA 19111, [2]Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL 35294.

Environmental xenoestrogens are endocrine disruptors that mimic the effects of endogenous hormones, and can therefore affect the development and function of reproductive organs. The xenoestrogen bisphenol A (BPA) is a plastic monomer commonly found in dental sealants, food cans and polycarbonate plastics. To better understand its possible influence in human health, we have studied the effects of neonatal and prepubertal exposure to this compound on the gene expression profile of the rat mammary gland during different stages of development. Pregnant Sprague Dawley rats were maintained on AIN-93G diet (phytoestrogen-free). Litters were exposed from birth (day 1) until weaning (day 21) to BPA through the milk of treated dams. Lactating mothers were gavaged daily (Monday through Friday) with 250 μg BPA/kg body weight or with an equivalent volume of sesame oil (controls). On days 21, 35, 50 and 100 of life, ten female offsprings from each group were euthanized. Total RNA from the abdominal mammary glands was extracted and individually quality verified. The RNAs were pooled and reduced to three samples per group. Samples were fluorescently labeled for hybridization to Agilent 60-mer oligo microarrays containing 22,000 features. Image analysis was performed using Feature Extraction and Imagene softwares; data were analyzed using GeneSight software and normalized by Lowess method. Only genes with ≥1.4-fold differences at $p<0.05$ confidence analysis were considered. Expression profile in the mammary glands of the 21 day-old rats showed upregulation of 70 genes, which included adhesion molecule genes as rCdh8, proteins related to cell proliferation and differentiation as WT1 and FABP3, and 53 unknown proteins. Down-modulation of GAD1 was also found. At 35 days of age 3 unknown genes were upmodulated and 3 were downmodulated. At 50 days there was significant upregulation of 127 genes and downregulation of 11 genes, including the inhibitor of apoptosis Birc2, participants of metabolism, signal transduction (Pkib, PKC theta), and proteins related to immune functions (complement component C3, cathepsin E, Scya2, Scya17) as well as 96 unknown genes. By 100 days of age the mRNA levels of 4 genes was increased, while 17 genes had decreased expression, being the majority of them unknown. The only gene expressing changes at all ages was the downmodulation of GAD1, a gene that has been reported to be overexpressed in several human and animal tumors. The level of expression of rCdh8, WT1, FABP3, Birc2, C3, cathepsin E, Scya2, Scya17 and GAD1 was confirmed by quantitative real time reverse transcription PCR. These analyses indicated that exposure to bisphenol A during the lactational period influences the expression pattern of the mammary tissues as a function of age, except for GAD1 gene, which was significantly downregulated in the mammary gland in all the different ages studied. (Work supported by NIEHS Grant U01 ES012771)