The Effect of Prepubertal Exposure to Bisphenol A on Rat Mammary Gland Morphology and Gene Expression

Zachary Rotter; ***Julia S. Pereira; Ricardo Lopez; Fathima Sheriff; Kara Snider and Jose Russo***

Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, PA, 19111, USA.

Abstract

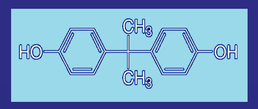
Bisphenol A (BPA), formerly called 4,4'- dihydroxy- 2,2-diphenylpropane, is a commonly used chemical found in re-microwaveable baby bottles and the polycarbonate lining of plastic bottles. BPA is found in normal levels in the environment, mostly as a byproduct of industrial processes. Studies have shown that the levels of BPA found in the environment are non-hazardous to human health. However, research also suggests that BPA could lead to breast cancer since BPA is an endocrine disruptor, meaning it can mimic hormones that have a functional role in the body. Due to these properties it was of interest to determine whether BPA could alter the terminal end buds (TEBs) structures of the mammary gland that are the target of carcinogenesis. For this purpose we have analyzed the effects of prepubertal exposure to BPA on the 50 days old rat's mammary gland.

Nursing rats received, through gavage, 250 µg/kg body weight of BPA, during 21 days, from the delivery to weaning. The female offspring were sacrificed when they reached 50 days of age. Their abdominal mammary glands were extracted and used for whole mount preparation for TEB counting, or cDNA-microarrays gene expression analysis. The results indicated a slight decrease in the number of TEBs in mammary glands of the treated rats when compared to the control group. However, gene expression analysis revealed changes in expression of numerous genes reported in breast cancers, such as Vav2, Nfkb1, Tnsrf11a, and Mycn. We have concluded that although prepubertal exposure to BPA does not affect significantly the rat mammary gland morphology of 50 day old rats when compared to the control group, the compound has an effect on the mammary gland gene expression. BPA altered the expression of some genes that have been reported in primary breast cancer as well as in the inflammatory type.

Introduction

Phthalates are among the most widespread environmental pollutants today, largely because organisms can be exposed to them through ingestion, inhalation, and dermal contact. Due to their lipophilic nature, phthalates pose bioaccumulation risks; repeated exposure to even small amounts of these compounds can cause serious adverse health affects. Phthalates may be passed to offspring through lactation, and in turn adversely effect the developmental morphology and gene expression of *Rattus Norvegicus* mammary glands, possibly facilitating a micro-environment hospitable to the development of breast cancer. It is hypothesized that BPA action on the mammary gland is due to its xenoestrogenic nature. As only approximately 10% of all breast cancer cases are linked through family genetics, the investigation of the possible effects of BPA in terms of mammary gland carcinogenesis is vital to further understand and treat this widespread disease.

BPA is commonly used as a structural compound found in the polycarbonate linings in various plastic products. Examples of this are re-microwaveable baby bottles, re-useable water bottles, recyclable water bottles, and stainless steel appliances. The weak bond between BPA and the polycarbonate lining poses the risk of BPA ingestion; with each repeated use, ingestion risks increase as well.



Bisphenol-A (BPA)

Acknowledgements

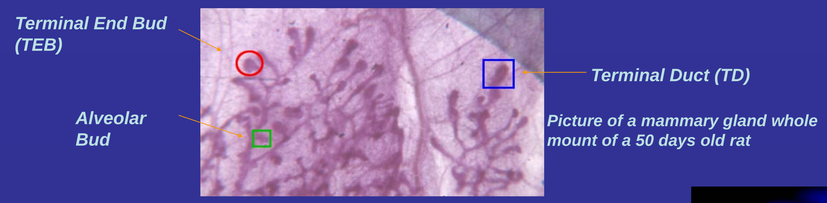
Work supported by NIEHS Grant U01 ES012771

Zachary Rotter was sponsored by the Huntington Breast Cancer Action Coalition through the Students and Scientist Environmental Research Scholarship Program.

Methodology

Structural Analysis Procedures

–Whole mounts of the rat mammary gland were prepared using Alum Carmine staining.
–Terminal end buds were counted.
–The resulting data were analyzed using two-tailed unpaired t-test for statistical comparison.



Microarray Analysis Procedures

–Comparison to the control group showed whether a gene was over-expressed or under- expressed by the specific treatment.
–Genes with p<0.001 were considered differentially expressed.
–A literature search was done to determine which of these genes have documented links to mammary carcinogenesis.

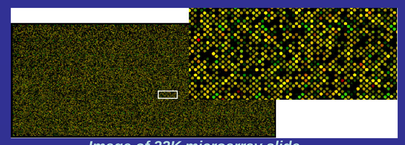


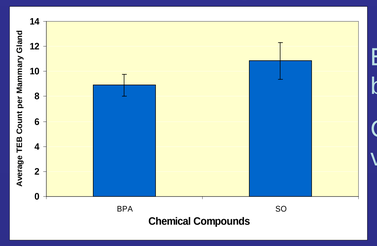
Image of 22K microarray slide

Picture of Agilent 1" x 3" glass slide

cDNA Microarray Analysis

Results

Structural Analysis



BPA Group: 250 µg of BPA/ kg of body weight

Control Group (SO): equivalent volume of sesame oil

	BPA	Control
Mean TEB Count	8.9	10.9
Standard Deviation	3.8	4.6
Standard Error	0.27	1.5
Range	5-20	6-15
P-value (T-test)	0.27	

Gene Expression Analysis

- 271 differentially expressed genes were found to be deregulated by BPA.
- Of the 271, 138 were upregulated, and 133 were downregulated.
- Gad1 had the highest downregulation compared to control (8 times down)
- Usp29_predicted had the highest upregulation (4 times up)

Genes found to be modulated by BPA and also reported to be related to Inflammatory Breast Cancer:

Tcf12: Upregulated in T-cell and B-cell lymphocytes
Nfkb1: Involved in angiogenesis, cell proliferation, inflammation
Tnf, Tinf2 and Tnfrsf11a: Immune response, codes for inflammation.
Vav2 and Mycn: Oncogenes

Conclusions

- Morphological analysis demonstrated that the mammary glands of rats treated with BPA did not have substantial changes in the number of TEB compared to the control group.
- In the cDNA microarray analysis, there were several differentially expressed genes in BPA treated rats which may encourage an environment hospitable to the development of breast cancer, such as Vav2, Nfkb1, Tnsrf11a, Mycn and Rabl3.
- Notably, some of the genes modulated by BPA have been also found to be deregulated in Inflammatory Breast Cancer.