Effect of Prepubertal Exposure to Environmental Contaminants in the Rat Mammary Gland

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Students & Scientists Environmental Research Scholarship Program

Huntington Breast Cancer Action Coalition

Abstract

Background

Over the course of one month, we had the opportunity to participate in the BCRL through "The Students and Scientists Environmental Research Scholarship" sponsored *by Prevention Is The Cure,* Inc., a campaign of the Huntington Breast Cancer Action Coalition (HBCAC).

Objective

The aim of this internship was to learn how scientific research was conducted through studies on the effects of environment contaminants in the rat mammary gland.

Work performed

To accomplish our objective we studied the morphology and the effects of prepubertal exposure to Bisphenol A (BPA) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), two well known endocrine disruptors (ED) in mammary glands. For our experiment we used whole mounts (WM) and tissue sections of mammary glands, collected and prepared at a collaborating institution. Lactating rats were treated either with solvent (control groups), BPA or TCDD. The treatments were given as follows, either: a daily intragastric administration of BPA for 21 days; or TCDD when the pups were 14 and 17 days of age. Tissue was collected from female offspring of all groups at 50 days of age.

First, we learned about the mammary gland structures, by counting the number of terminal end buds (TEBs) from previously prepared WMs. Secondly, we learned how to recognize the cell division phases, counting cells that were in metaphase and anaphase, in 500 epithelial cells from hematoxylin and eosin stained sections. Ten slides per group were counted and the treatments were maintained blinded. Thirdly, to assess the interpersonal variation the individual results from both groups were counted by each of us and compared. Lastly, we learned how to use the student t-test to assess the statistical significances of the data.

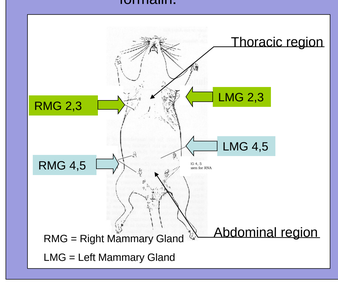
Conclusions

Through this experience, we gained an understanding of the research process and the significance of environmental contaminants to human health. We understand that it is possible that early exposure to hormonally active environmental compounds may affect the architecture and cell division of the rat mammary gland during critical stages of development. These effects could facilitate the occurrence of mutations and eventually initiation of cancer.

Materials & Methods

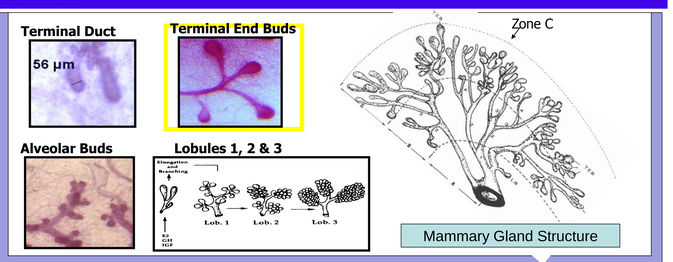
Sample Collection

- Animals are weighed and anesthetized with ketamine (90mg/Kg)
- Mammary glands 2,3 (thoracic) and 4,5 (abdominal) are removed.
- Other organs that are collected include: uterus and ovaries, spleen, pancreas, liver, kidney, heart and lungs.
- Samples are immediately frozen in liquid nitrogen, place in dry ice or fixed in 70% ethanol or 10% formalin.



Hematoxylin and Eosin Staining

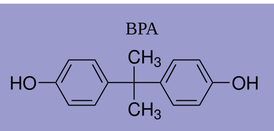
- Hematoxylin is a blue nuclear stain, hence staining the DNA and nucleus of the cell
- Eosin is a pink stain that stains the cytoplasm, connective tissue and other extracellular substances of the cell
- The tissue sample must be fixed in 70% ethanol (8-10 hours) or 10% formalin (18-24 hours) and passed through a modular vacuum processor, and undergo tissue embedding and sectioning beofre it can be stained



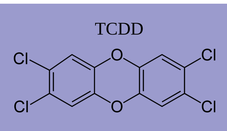
Whole Mount Preparation

- The mammary gland is fixed in either 10% formalin (18-24 hours) or 70% ethanol (8-10 hours).
- They are then stored in acetone and stained with alum carmine (a purple nuclear stain) overnight.
- Lastly, the sample is dehydrated using increasing ethanol concentrations (70% to 100%) and stored in Histoclear solution overnight before being mounted on a glass slide using permount.

Background



- An important building block for several plastics and plastic additives.
- This compound can mimic the human body's own hormones and acts as estrogen.
- BPA is found in water and baby bottles, sports equipment, medical devices, dental fillings and in many other areas.
- Used primarily in production of polycarbonate plastic.

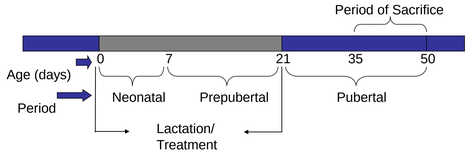


- Polychlorinated dibenzodioxins are some of the most toxic and abundant forms of environmental pollutants.
- Byproducts of industrial waste incineration, incineration of chlorine-containing substances, and fuel combustion.
- Lipophilic properties allow dioxins to bio-accumulate in humans.
- 2,3,7,8-tetracholorodibenzo-p-dioxin is the most toxic of the dioxin family.
- TCDD has both xenoestrogenic and antiestrogenic effects in various biological environments.

Treatment

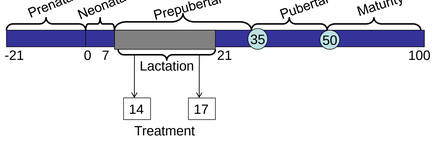
BPA

- Control – Equivalent volume of sesame oil
- BPA – 250 µg/kg body weight
- BPA is given to the mother by gavage for 21 days and the pups receive the treatment through lactation

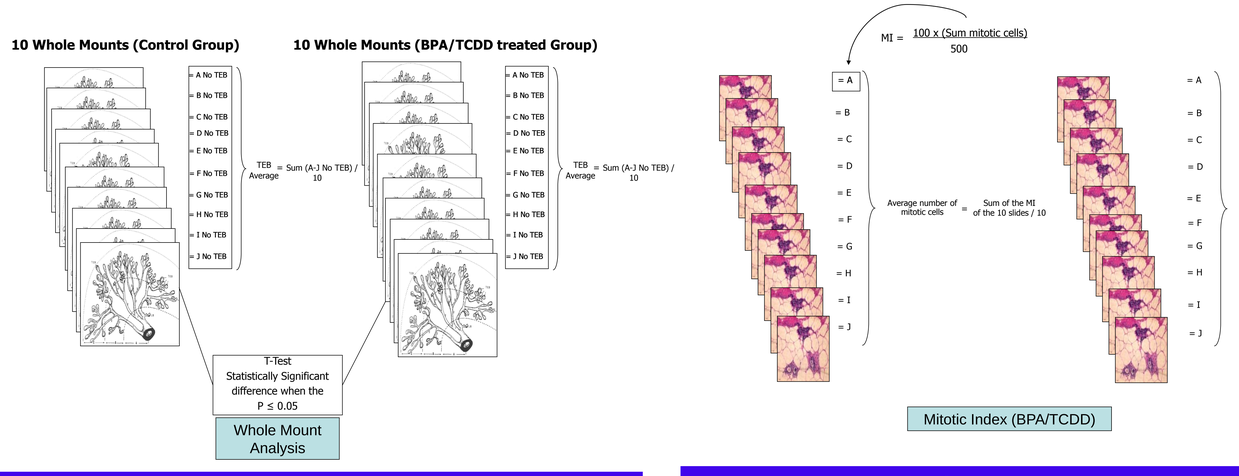


TCDD

- Control – Equivalent volume of sesame oil
- TCDD – 20.0 ngTCDD/g BW
- TCDD is given to the mother by gavage over a two day period (day 14 & 17). The pups are exposed to the treatment by lactation.



Results



Significance of environmental contaminants to human health

BPA	TCDD
•Recurrent miscarriage •Altered mammary gland development •Altered brain development •Insulin resistance	•Skin Lesions •Impairment of the immune system, nervous system, and endocrine system •Impairment of reproductive functions

Conclusions

- We understand that it is possible that early exposure to hormonally active environmental compounds such as BPA and TCDD may affect the architecture and cell division of the rat mammary gland during critical stages of development. These effects could facilitate the occurrence of mutations and eventually initiation of cancer.
- A further understanding of the effects of chemical compounds on the development of mammary glands is crucial so that possible carcinogens such as BPA can be correctly identified as harmful, and steps can be taken in society to limit human exposure to such chemical compounds.

Reducing Our Exposure

BPA	TCDD
•Cut back on consumption of canned foods •Avoid heating foods in polycarbonate containers •Before getting dental sealants, check with your dentist about the ingredients in the products	•Screening tests in high-risk areas (i.e. industrial processes & waste incineration) •Limit exposure to wastes from industrial processes. •Check the components of all pesticides that you may come in contact with.

Acknowledgements

•Dr. Jose Russo and our mentors Fathima Sheriff, Johana Vanegas, Lucas Bidinitto and Kara Snider
•The Fox Chase Cancer Center at Philadelphia and its staff.
•The Huntington Breast Cancer Action Coalition (HBCAC)
(Ansari and Fishstein were supported by HBCAC and this work was supported by grant U01 ES/CA 12771 from NIEHS and NCI)

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