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

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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 & 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 environmental contaminants in the rat mammary gland.

Work performed
To accomplish our objective we studied the morphology and 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 and 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 interindividual 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 understood 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 anaesthetized with ketamine (50mg/kg)
• Mammary glands 2.3 (thoracic) and 4.5 (abdominal) are removed.
• Other organs that are collected include: udders and ovaries, spleen, pancreas, liver, kidney, heart and lungs.
• Samples are immediately frozen in liquid nitrogen, placed in dry ice or fixed in 70% ethanol or 10% formalin.

Sample Preparation
• Hematoxylin and Eosin Staining
• Paraffin embedded tissue is sectioned at 5um thick, hematoxylin - eosin stained
• The tissue sample must be fixed in 70% ethanol (8-10 hours) or 10% formalin (18-24 hours) and passed through x modular vacuum processor, and undergo tissue embedding and sectioning before it can be stained

Significance of environmental contaminants to human health

- 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.

- Polybrominated dibenzodioxines are some of the most toxic and abundant forms of environmental pollutants.
- BPA is found in water and baby bottles, sports equipment, medical devices, dental fillings, and in many other areas.
- BPA is used primarily in production of polycarbonate plastic.

- This compound can mimic the human body’s own hormones and acts as estrogen.
- BPA has been linked to a variety of health problems, including fertility issues, obesity, diabetes, and increased risk of certain cancers.

- Hematoxylin and eosin staining is a common method used to identify structures within tissue samples.

- Paraffin embedding and sectioning are used to prepare tissue samples for further analysis.

- The tissue samples are fixed in 70% ethanol or 10% formalin, which helps preserve the tissue and DNA for subsequent analysis.

- The tissue samples are then passed through a modular vacuum processor, which helps to remove residual moisture and ensure uniformity.

- Tissue embedding and sectioning are performed to prepare the tissue samples for microdissection and staining.

- The tissue samples are then stained with hematoxylin and eosin, which helps to distinguish different cell types and structures.

- The stained tissue samples are then analyzed under a microscope to identify and characterize the structures.

- This process is crucial so that possible carcinogens such as BPA can be correctly identified as harmful, and steps can be taken to reduce exposure.

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 as this is where all 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.

References

Reducing Our Exposure

- Cut back on consumption of canned foods
- Avoid eating foods in polycarbonate containers
- Before giving prenatal exams, check with your dentist about the ingredients in the products.

Acknowledgements

- Dr. Joseph Russo and our mentors Fathima Shehiff, Jehana Vanegas, Lucas Bidinotto and Kara Sider
- The Fox Chase Cancer Center at Philadelphia and its staff
- The Huntington Breast Cancer Action Coalition (HBCAC) (BPA and Bisphenol A were supported by NIEHS and NIOSH)

TCDD
- bowel, brain, breast, cervix, endometrium, fibrocystic, gallbladder, heart, kidney, lung, ovary, prostate, skin, testis, uterus
- Adverse effects may include cancer and reproductive disorders.