

In vivo impact of xenoestrogen exposure on the human breast

Investigators

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Research Program

California Breast Cancer Research Program

Research Priority

Community Impact of Breast Cancer

Award Type

Community Research Collaboration Award

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24BB-2200

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\$788,220

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Abstract

Introduction: Manufactured chemicals, such as phthalates and parabens, found in many consumer products are mimics of the natural hormone estrogen. Exposures to these "xenoestrogens" ("XEs") have been linked to mammary cancer in rodents and shown to cause pre-cancerous changes in normal human breast cell cultures grown in the laboratory. Our recently-completed pilot study tested the feasibility of measuring the impact of decreased XE exposure from personal care products (PCPs) on normal human breast cells while still in the human body by comparing cells donated by volunteers through fine needle aspiration (FNA) before and after a healthy intervention requiring use of XE-free personal care products. Breast cancer survivors successfully recruited and supported study volunteers, resulting in 100% retention through two FNA procedures and full compliance with study protocol. Significant reductions in cellular features that precede the genesis of breast cancer were observed. This study aims to validate pilot study findings by increasing the number and diversity of study participants, adding a wait-list control group that will not participate in the XEL intervention, and including a carcinogenesis-relevant gene expression profile to the biological features studied in the cultured cells. The study aims to provide key insights into the biological processes of cancer

development by studying the role of XEs in cellular changes that precede the genesis of breast cancer. Question(s) or hypotheses: We hypothesize that an intervention that decreases exposure through use of XE-free PCPs will reduce gene expression and cellular changes in breast tissue that are associated with cancer development. General methodology: We will recruit 60 women, 18-45 years of age, who use PCPs containing xenoestrogens. Study participants will contribute urine and blood samples and donate breast cells through FNA performed by an experienced breast surgeon. By random selection, 40 of the participants will be provided with a kit of approved phthalate and paraben-free PCPs to use during the intervention period to decrease XE exposure, and 20 participants, acting as a control group, will continue to use their regular products which contain XEs. After 28 days, each participant will again contribute FNA, urine, and blood samples. Urine will be analyzed for levels of phthalate and paraben breakdown products, blood will be analyzed for base levels of natural hormones, and breast tissue will be cultured in the laboratory and analyzed for specific cellular features associated with the initiation of breast cancer. Additionally, FNA samples will be profiled for changes in gene expression underlying cell-based tests. Study data will be analyzed to determine if the 28-day use of XE-free products reduces XE exposure as shown by levels in urine, and whether it impacts cancer-related features and gene expression within donor breast cells. Innovative elements: Recruitment of study volunteers will be conducted entirely by breast cancer survivors, with outreach to specific community groups to increase racial diversity of volunteers. Survivors will provide personal support to study volunteers, using pilot study methods improved by participant feedback. Participants will benefit from increased awareness of XE-exposures, changed PCP use, and satisfaction from their contribution. The full study's unique combination of in vivo/in vitro analysis of changes in normal human breast cells has great potential for use in studies of other environmental chemical exposures. Community involvement: The impetus for this study came from a group of breast cancer survivors seeking to promote breast cancer prevention by supporting research into the effects of exposures to estrogenic chemicals found in consumer products on the genesis of breast cancer. These community members found innovative and experienced cancer researchers to address their research question, and initiated and participated in a CRC pilot study. The pilot demonstrated their ability to recruit women from the community to provide normal breast cells for scientific research, as well as the feasibility of using donated cells to study human breast carcinogenesis. Their active involvement will continue through the full study as they recruit, support and educate study participants, assist in data analysis, and translate and publicize results. Future Plans: The data obtained through this study will be used to promote breast cancer prevention through decreased XE exposure. Additionally, the research model first tested in the pilot study and intended to be validated and expanded through the full study will be invaluable for the design and implementation of future studies on the impact on normal cells of exposures to a variety of environmental chemicals that may contribute to human breast carcinogenesis.

Progress Report Abstract

Estrogenic overstimulation is carcinogenic to the human breast. To identify the effects of persistent exposure to xenoestrogens (XEs), such as phthalates and parabens in personal care products (PCPs), directly within the human breast, we developed a Community-Research Collaborative. Together, we designed and implemented an XE Low Intervention (XELINT)

protocol. Pre- and post-intervention fine needle aspirates (FNAs) of healthy breast tissue were collected from individuals who volunteered to use a study-provided phthalate and paraben-free PCP kit over a 28-day menstrual cycle, and from Control subjects who continued with their usual PCP choices. Despite unexpected obstacles in subject recruitment due to the COVID-19 pandemic, project aims were executed with 41 in-hand sample sets. The successful identification of molecular differences between pre- and post-intervention FNAs was possible using a matched subset. Notably, when study-provided PCPs were used, a striking reversal from 'cancer-prone' to 'normalized' expression levels of genes representing major molecular pathways was observed. Significant changes ($p < 0.01$) involved genes in the breast cancer-associated mTOR/PI3K-AKT pathway of cell survival signaling, found to be activated in nonmalignant breast cells by another common XE – bisphenol A (Dairkee et al., *Cancer Research* 68:2076, 2008). Gene expression changes in breast cells were significantly correlated with reduced urinary paraben metabolite levels. Additionally, ex vivo exposure of FNA-derived live breast cells to the natural estrogen - 17 β -estradiol (E2), also displayed a 'normalizing' impact of XELINT on E2-regulated genes. Project highlights include: (a) Participant adherence to study protocols and return visits, (b) Zero attrition in the acquisition of matched pre- and post-intervention samples from recruited volunteers (c) Seventy percent success rate for matched FNA propagation and RNA collection allowing sufficient statistical power to measure significant differences between subject groups. (d) Gene signature representing the in vivo impact of reducing phthalate and paraben intake through daily-use PCPs. Our study reveals unfavorable consequences to humans of the continuous intake of 'safe' XE levels from PCPs. Insights from the novel approaches implemented here ensure the feasibility of future confirmatory studies. Importantly, this research demonstrates the potential for overturning insidious pro-carcinogenic phenotypes induced by XE exposure towards the goal of breast cancer prevention.

Publications

Title	Authors	Year
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