Effects of Oxybenzone on Epithelial Ductal Development in Murine Mammary Glands

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Background

Oxybenzone (benzophenone-3; BP-3) is a putative endocrine disrupting chemical, and common ingredient in sunscreens and many personal care products. Endocrine disrupting chemicals can interfere with the normal action of reproductive hormones. BP-3 is found in the urine of as much as 98% of the U.S. population. Given the prevalence of BP-3 exposure, published evidence that BP-3 is an endocrine disrupting chemical, and the lab's previous studies showing that a high-fat diet can promote breast cancer, we investigated the effects of BP-3 and diet on the ductal development of mammary glands in mice. Preliminary findings suggest the withdrawal of BP-3 after lengthy exposure can induce regression of mammary gland development. The results also indicate that diet has an impact on the development of mammary glands.

Method

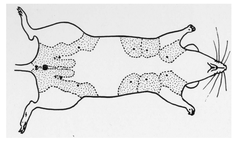
Diet

BALB/c mice were used due to their resistance to obesity. This allowed for the effects of BP-3 and diet to be assessed without the confounding effects of obesity. BALB/c mice were fed diets with differing levels of dietary fat in the form of lard, a source of saturated animal fat; low-fat (LFD; 10% kcal fat) or low fat switched to high fat (HFD; 60% kcal fat) with the change occurring at puberty (10 weeks). Varying dietary treatments of BP-3 (70 mg/kg of body weight) also occurred for each diet group

- No treatment
- Continuous treatment
- Withdrawal 2 weeks prior to experiment termination
- Withdrawal 4 weeks prior to experiment termination

Whole Mount

At 26 weeks, the inguinal mammary glands were collected. The mammary glands were fixed with formalin, stained with carmine, and dehydrated by ethanol and xylene. The whole mounts were then stored in methyl salicylate.

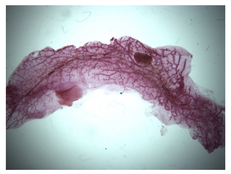


Microscopy

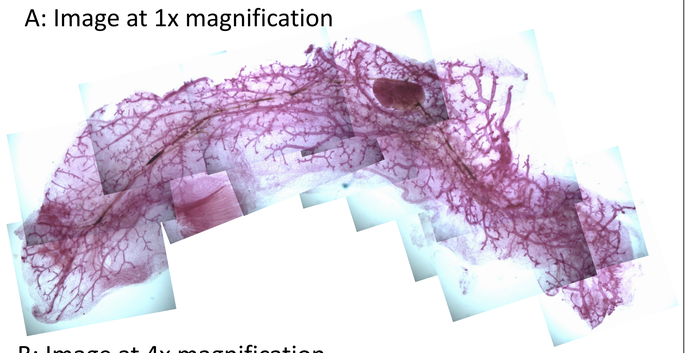
The whole mounts were examined under a NIkon SMZ-2T stereo microscope at 4x magnification. 4x magnification was determined to be the lowest magnification that could be used while allowing for identification of branch points. Images were taken at this magnification using QCapture Pro 7 imaging software and were compiled into a complete image of the gland. A 240 square grid was overlaid upon the image of the mammary gland. The number of branch points in each square containing tissue was calculated to determine frequency of branch points in each gland

Mammary Gland Morphology

Low-Fat Diet

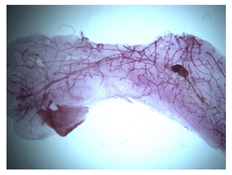


A: Image at 1x magnification

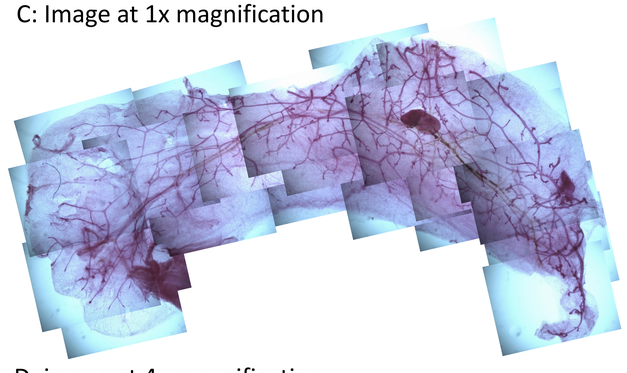


B: Image at 4x magnification

Low to High-Fat Diet



C: Image at 1x magnification



D: image at 4x magnification

Figure 1: Whole mount of 26 week mouse mammary gland at 1x and 4x magnification
At 4x magnification, the ductal development of image A, representing the low-fat diet group, can be seen to be more developed and have greater complexity than the ductal network of image D, representing the low to high-fat diet group.

Results

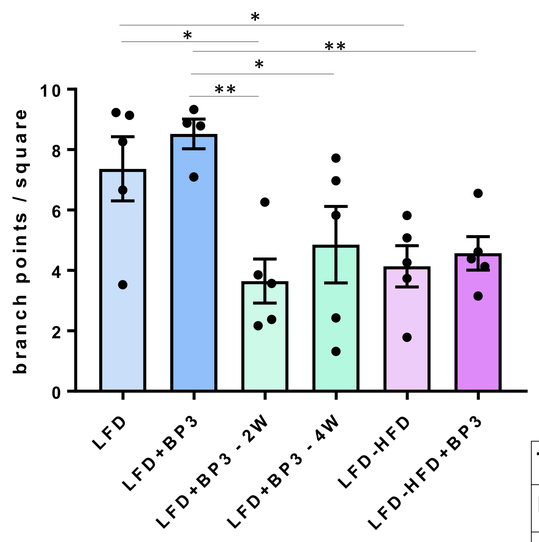


Figure 2: Branch point frequency of 26 week BALB/c mice
T-Tests indicate significant effects between LFD +/- BP-3 and withdrawal groups, especially two week withdrawal. There is also a significant difference between LFD +/- BP-3 and LFD-HFD +/- BP-3 groups indicating an effect of diet.

* $p \leq 0.05$
** $P \leq 0.01$

Two-Way ANOVA	
BP-3	0.3147
Diet	0.0003
Interaction	0.6409

Table 1: Two-way ANOVA between LFD +/- BP-3 and LFD-HFD +/- BP-3 groups
The effect of diet between these four groups was significant with p = 0.0003. Analysis has not yet been completed for the LFD-HFD + BP3 – 2W and LFD-HFD + BP3- 4W treatment groups.

Conclusions

BP-3 Withdrawal Effect: There is a significant effect of withdrawing BP-3 on the development of mammary glands. Both the two and four week withdrawal were significantly different from continuous BP-3 treatment (p = 0.0012 and p = 0.0442, respectively). The two week withdrawal group was significantly less developed than the low-fat diet without BP-3 treatment (p = 0.0205). These results indicate BP-3 has an impact in mammary gland development and future studies are needed to determine the mechanism underlying the regression of ductal branching that occurs with BP-3 withdrawal. Furthermore, these findings suggest BP-3 as an endocrine disrupting chemical and call for more study of the health impacts of this environmentally prevalent chemical.

Diet Effect: There is a significant impact of diet on mammary gland development. Switching from low-fat to high-fat diet at puberty resulted in a significantly less developed mammary gland, regardless of BP-3 treatment. A significant effect of diet was found between the LFD +/- BP-3 and LFD-HFD +/- BP-3 groups (2-way ANOVA p =0.0003). These results indicate an effect of diet on mammary gland development and future studies are needed to better understand the mechanisms underlying the high-fat diet suppression of ductal branching.

Acknowledgements

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