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Eileen A. Holovac

University of Rhode Island, eileen_holovac@my.uri.edu

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Evaluation of BDE-47 and -99 lipid modulating effects in HepG2 human carcinoma cells

Eileen Holovac, Prajakta Shimpi, Angela Slitt
University of Rhode Island College of Pharmacy

Background
Non-alcoholic fatty liver disease (NAFLD) is becoming a significantly more common problem in today's society, affecting up to 25% of people in the United States as reported by the American Liver Foundation. According to the American Association of the Study of Liver Diseases, NAFLD is the buildup of fat in the liver that is not caused by secondary factors such as alcohol consumption, hereditary disorders, or the use of steatogenic medication such as amiodarone. A liver is considered fatty when 5-10% of the liver's weight is fat. The progression of NAFLD can lead to cirrhosis, liver cancer, or liver failure. Risk factors for NAFLD include obesity, type 2 diabetes mellitus, hypertriacylglycerolemia, hypercholesterolemia, age, gender, and ethnicity. In addition, there are examples of toxicant-induced liver disease in occupationally exposed workers, suggesting that the environment may also be a risk factor for the development of NAFLD. This study aims to determine whether direct exposure to environmental compounds cause fatty liver using cultured liver carcinoma cells.

Introduction
BDE-47 (2,2′,4,4′-tetra-bromodiphenyl ether) is a brominated flame retardant used in a wide variety of consumer products such as polyurethane foam, which is used in furniture and car upholstery, packaging and electronic equipment. BDE-47 is released into the environment by manufacturers and by the products themselves and can be ingested or inhaled and then stored in the liver as lipids. The pentabDE congener that is usually predominant in environmental media is BDE-99 (2,2′,4,4′,5-penta-bromodiphenyl ether). BDE-99 is a brominated flame retardant chemical and is released into the environment. PentabDEs are thought to be distributed through the human body and found in adipose tissues, blood, liver, and maternal milk. My hypothesis is that BDE-47 and BDE-99 will increase the total lipid content in cultured HepG2 liver carcinoma cells.

Hepatic Lipid Homeostasis

Hepatic Steatosis Progression

HepG2 Cells:
HepG2 cells are a human liver carcinoma cell line derived from a 15-year-old Caucasian male. HepG2 cells are a good model for studying NAFLD because they have morphological and functional differentiation which accurately represents human liver cells.

Procedural HepG2 cells grow and passaging, seeding plates:

Methods and Materials

Triglyceride quantification:

Data/Results

Figure 1: A. Total lipid accumulation in HepG2 cells was measured after treatment of vehicle or different doses of BDE-47 and 99 along with oleic acid. After 24hrs of treatment total lipids were extracted and dried in a glass tube using a speedvac. The weight of the lipid pellet was noted. Total lipid content is represented as normalized to control group and one-way ANOVA followed by Dunnett's test was applied to compare treatment exposure versus control group.

B. Triglycerides from the solubilized lipid pellet were quantified by calorimetric methods. The triglyceride content is represented as normalized to control group and one-way ANOVA followed by Dunnett’s test were applied to compare BDEs exposed versus control group.

Oleic acid co-treatment increased lipid content. 0.1 mM BDE 47 increased lipid content in HepG2 cells, in agreement with total lipids. TG levels also increased with 5mM BDE 47 treatment. Oleic acid co-treatment increased TG content in 1 and 25mM BDE 47 and 1mM BDE 99.

Conclusion
BDE exposure may increase the risk of non-alcoholic fatty liver disease. BDE 47 showed the most prominent effects on HepG2 cells leading to hepatic steatosis.

Future Plan
Future research will be conducted on the mechanism of BDE-induced non-alcoholic fatty liver disease. We will be researching other genes that may be involved in BDE-induced non-alcoholic fatty liver disease through the use of Real-time PCR. We will also be using different time frames for BDE treatment.

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