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

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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 II diabetes mellitus, hypertriglyceridemia, 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.

Hepatic Lipid Homeostasis
Dietary fat is a major source of lipid intake. Dietary fat is absorbed in the small intestine and transported in the blood as triglycerides. Triglycerides are then stored in adipose tissue or used for energy. When adipose tissue is full, excess triglycerides are 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 Steatosis Progression
Non-alcoholic fatty liver disease (NAFLD) is a common liver disease characterized by an abnormal accumulation of triglycerides in the liver. NAFLD can progress to non-alcoholic steatohepatitis (NASH), which is associated with an increased risk of liver cirrhosis and liver cancer. Long-term NAFLD is a significant risk factor for the development of liver cancer.

Methods and Materials

HepG2 Cells: HepG2 cells are a human liver carcinoma cell line derived from a 15-year-old Causcasian male. HepG2 cells are a good in vitro model system for this study because they have morphological and functional differentiation which accurately represents human liver cells.

Procedure of HepG2 cells grow and passaging, seeding plates:

- Grow HepG2 cells in T-25 flasks (D-MEM + 10% FBS + 1% N-A + 1% NaN3)
- Place cells in 50 mm plate
- Total cells with different components
- Harvest cells and split

Composants Protein concentration Working stock (100x)

- Albumin 10 mg/ml 1000 mg
- FAS 0.1 mg/ml 10 mg
- Oleic acid 1 mg/ml 10 mg
- TG 1 mg/ml 10 mg

Data/Results

Total Lipids

Total triglycerides

Triglycerides

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.

SREBP-1C

Relative cell lipid expression

SREBP-1C was significantly increased in BDE-47 treated HepG2 cells. Unexpectedly, 1mM BDE 47 decreased SREBP-1C mRNA expression.

BDEs

Pre-dispose 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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