DOCTORAL THESIS

Pathological effects of persistent organic pollutants on obesity and obesity-related liver diseases

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ABSTRACT

The worldwide prevalence of obesity and obesity-associated liver diseases have attracted great attention in the past decades. Obesity is an increasing health problem, which can induce a series of metabolic syndrome associated diseases, such as fatty liver disease, type 2 diabetes. The conventional causes for obesity, such as over-eating, sedentary life-style, and genetic factors, cannot fully explain the global rapid increase of obese population in the last few decades. It was found that the production of persistent organic pollutants (POPs) in the industry was closely correlated with the prevalence of obesity. POPs are organic chemicals that are resisted to degrade by various processes and widely applied in daily products to improve the quality of our life. 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) is the most abundant and toxic congener in the family of polybrominated diphenyl ethers (PBDEs), which are the commonly used flame retardants and listed as POPs in 2009. High concentration of BDE-47 has been found in indoor dust and marine fish in Hong Kong. Owing to their high lipophilic and persistent characters, BDE-47 is mainly accumulated in adipose tissue. Epidemiological data indicates that exposure to BDE-47 is associated with obesity and obesity-associated liver diseases. Therefore, based on published research, we hypothesize that BDE-47 exposure may increase the occurrence of obesity and aggravate the progression of obesity-associated fatty liver disease through promoting adipocyte differentiation and impairing lipid metabolism.

To verify this hypothesis, mouse preadipocytes (3T3-L1 cells) were exposed to BDE-47 and differentiated into adipocytes. Excitedly, with BDE-47 exposure, more lipid droplets were formed and accumulated in the treated cells than that in

untreated adipocytes (without BDE-47 exposure). Along with the increased content of triglyceride accumulation, augmented gene and protein levels of transcription factors (PPARγ and PGC-1α), and related genes (FABP4 and C/EBPα) were also detected in BDE-47 treated cells. In addition, the total production of reactive oxygen species (ROS), contents of lipid peroxidation and DNA oxidation were obviously increased in adipocytes treated with BDE-47 (10 μM). To explore how BDE-47 regulated the oxidative stress signal pathways, antioxidants of ROS sources were employed with BDE-47 exposure during adipocyte differentiation. Notably, mitochondrial respiration, xanthine oxidase and NADPH pathway were significantly influenced by BDE-47 exposure to generate ROS in the treated adipocytes. The effects of BDE-47 on mitochondrial respiration were also determined for further exploring the relationship between mitochondrial ROS and adipocyte differentiation. Significant elevation of mitochondrial ROS was detected in adipocytes exposed with BDE-47 (10 μM). Furthermore, to support the energy requirements for the growth of adipocytes during differentiation process, BDE-47 improved the mitochondrial metabolism for ATP production via increasing the spare mitochondrial respiration capacity. Inhibiting the mitochondrial ROS generation in BDE-47-treated adipocytes with antioxidant attenuated the generation of ROS and reduced the accumulation of lipid droplets as well. This phenomenon indicated that the ROS-induced by BDE-47 through mitochondrial chain was critical for adipocyte differentiation.

Global metabolomic profiling based on high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) was performed on differentiated 3T3-L1 cells to reveal the metabolic changes induced by BDE-47.

Twenty three significantly changed metabolites were identified in the adipocytes after BDE-47 exposure. The results of pathway analysis showed that purine and glutathione metabolism were the main impacted pathways and upregulated by BDE-47 treatment. In purine metabolism, increasing levels of adenosine monophosphate (AMP) and guanosine monophosphate (GMP) induced by BDE-47 led to the increment of inosine 5'-monophosphate (IMP) in adipocytes. These increases forwarded the pathway and caused high production of uric acid along with hydrogen peroxide, which contributed to the elevation of ROS after exposure to BDE-47. Inhibiting the synthesis of uric acid with antioxidant could significantly decrease the production of ROS, the levels of adipogenesis-related genes, and the accumulation of lipid droplets in BDE-47 exposed adipocytes. These results further demonstrated that exposure to BDE-47 promoted adipocyte differentiation via causing oxidative stress, upregulating purine metabolism, and increasing production of uric acid.

Subsequently, C57BL/6J mouse model with diet interaction was employed to explore the obesogenic effects of BDE-47. Male C57BL/6J mice were fed with either a low-fat diet (LFD, 10% fat) or high-fat diet (HFD, 60% fat) for 15 weeks and subcutaneously injected with BDE-47 (7mg/kg [Low dose, L] or 70mg/kg [High dose, H]) or the vehicle weekly. It was found that exposure to BDE-47 (H) significantly led to the elevation of body weight and serum triglyceride content in HFD fed mice. Besides, the combination of BDE-47 and HFD also significantly increased the weight of white adipose tissue (WAT) and augmented the size of adipocytes in WAT. These have confirmed the obesogenic effects of BDE-47 *in vivo*. Additionally, BDE-47 (H) exposure significantly increased the

accumulation of hepatic triglyceride content and lipid droplets accompanying with elevated inflammation in HFD fed mice, indicating the deterioration of hepatic steatosis in BDE-47 treated mice. Moreover, the integration analysis of lipidomic and gene expression revealed that BDE-47 up-regulated triglyceride synthesis but suppressed lipid exportation and β oxidation to impair the lipid metabolism and worsen the accumulation of hepatic lipid in HFD fed mice. In addition, the increase of liver fibrosis scars (the protein level of αSMA and collagens), serum transaminase levels, as well as lipid peroxidation have been detected in the mice with co-treatment of BDE-47 and HFD. BDE-47 exposure also increased the production of ROS and the levels of fibrotic genes in hepatocytes. However, in LFD with BDE-47 exposed mouse liver, we cannot observe such changes compared with the control (LFD-DMSO). Interestingly, the application of antioxidants reversed the BDE-47-induced fibrotic responses (the expression of αSMA and col3) in hepatocytes, which indicated that the increase of liver fibrosis scars was tightly associated with the level of oxidative stress. In conclusion, these results offered a new insight of lipid toxicities and underlying mechanism of BDE-47 induced obesity-related liver fibrosis.

As far as we know, this is the first systematic study of the obesogenic effects and underlying mechanisms of BDE-47 in diet-induced mouse model. These results have showed the pathological roles of BDE-47 in the development of obesity and related liver diseases by an integration analysis of omics study and biological analysis *in vivo* and *in vitro*. Meanwhile, inhibitors were applied to investigate the mechanism of BDE-47-induced toxicity. Taken together, our results indicated that BDE-47 exposure could accelerate the development of obesity and aggravate

the progression of fatty liver in obese mice via causing oxidative stress. This study may shed a light for an explanation for the worldwide prevalence of obesity and related liver diseases. Furthermore, this work reflects the potential of omics study and biological methods for toxicity assessment of environmental pollutants on human health. It would be helpful for the clinical diagnose and treatment.

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