EXPOSURE TO TETRABROMOBISPHENOL A ALTERS HEPATIC LIPID METABOLISM IN C57BL/6N MICE

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Introduction

Tetrabromobisphenol A (2,2-bis-(3,5-dibromo-4-hydroxyphenyl)-propane; TBBPA) is halogenated derivateves of bisphenol A (BPA), a typical xenoestrogen. This compound is used worldwide as a flame retardant in numerous products. TBBPA has been detected in sediment and mussels[1)], sewage sluge[2)] or air at the dismantling plant[3)]. Therefore, the human is exposed by TBBPA from various contamination sources such as outdoor and indoor air and foods *et. al.* TBBPA is also an endocrine-disrupting chemical that can be potentially human adverse effect. Actually, TBBPA has been detected in pooled human serum[4)], human milk[5)] and umbilical cords from mother-infant pairs[6)]. The evidence that perinatal TBBPA exposure could lead to alter metabolic features was provided by Tada *et al.* When TBBPA exposure to dams and offsprings of ICR mice, they found an increment in serum concentrations of total-cholesterol and liver weights[7)]. Thus, the studies with TBBPA as a human homeostasis disrupting factor are needed to better understand.

Peroxisome proliferator-activated receptor (PPAR) is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily. PPAR must form a heterodimer with RXR to bind DNA and activate transcription. PPAR has a large ligand binding pocket, and is unique in its overall tertiary structure. That provides easier access for ligands[8)]. There are three isotypes of human PPAR, called α, δ and γ[9, 10)]. In particular, there are two isoforms of PPARγ denoted as PPARγ1 and PPARγ2. Until recently their actions were thought limited to specific tissue types, having individual roles in lipid catabolism; peroxisome proliferation in the liver as PPARα[10)], or adipogenesis[11)] and macrophages[12)] as PPARγ. Although PPARδ is almost ubiquitously expressed[13, 14)], due to the lack of any selective agonists or antagonists, its roles have not yet to be ascertained.

Previous results demonstrated that TBBPA showed agonistic activity on specifically PPARγ1 and γ2, and further tended to promote the PPARγ target gene expression in HepG2. It was reported that TBBPA caused an increase in oxygen consumption in the presence of succinate, and indicating uncoupling effect in mitocondoria isolated from rat liver[15)]. In addition, we observed that TBBPA accumulated markedly in liver of mice. Thus, it is important to examine toxicity of TBBPA and define that mechanism in liver. To investigate the toxicity of TBBPA for hepatic lipid metabolism *in vivo*, we measured the lipid parameter and the expression of PPAR target genes in liver.

Materials and methods

1) *Animals*

Animal studies were conducted in accordance with Setsunan University institutional guidelines. Male C57BL/6N mice (8week old) were purchased from Japan SLC (Shizuoka, Japan). Animals had free access to water and were fed chow from Oriental Yeast Co. (Chiba, Japan). TBBPA (97%, Sigma, MO, USA) was completely dissolved in corn oil and administered orally at a volume of 10 mL/kg body weight. TBBPA (700 mg/kg body weight) was administered 3 for 2 weeks. Control animals were treated with the same volume of vehicle (corn oil). At the end of administration, mice were fasted for 16 hours and sacrificed under pentobarbital.

The livers were quickly removed and snap-frozen in liquid nitrogen, and stored at -80 °C for various measurements. Blood samples were obtained simultaneously for serum measurements.

2) *Blood lipid assays*

Serum triglyceride, total cholesterol, and high-density lipoprotein cholesterol (HDL) were determined using commercial assay kits (Wako, Osaka, Japan) according to the manufacturer's protocols.

3) *Biochemical analysis of liver tissue*

Total liver lipid was extracted from the liver using a protocol adapted from Folch *et. al*. Approximately 0.5 g frozen liver tissue was homogenized in 5 mL of 2:1 chloroform:methanol solution. The homogenate was put in a shaker for 1.5 h. Next, an additional 5 mL of 2:1 chloroform:methanol solution was added followed by 2 mL PBS, subsequently mixed and centrifuged at 2,000 rpm, 10ºC for 3 min. The aqueous layer was discarded and the tube flushed with nitrogen until the lipid pellet was dry. The tube containing the lipid was resolved by isopropanol containing 1% TritonX-100.

4) *RNA extraction and RT-PCR*

Total RNA was isolated from the mice liver using isogen reagent (Wako), following manufacturer's recommendations. Purity and concentration of the isolated RNA was determined by measuring the absorbance ratio at 260/280 nm and 230/260nm using a Nanodrop 2000 spectrophotometer (Thermo Scientific). RNA was reverse transcribed to complementary DNA (cDNA) using PrimeScript RT reagent Kit (TAKARA). Quantitative real-time PCR analyses were performed on a Thermal Cycler Dice Real Time System (TAKARA). The PCR master mixture contained KAPA SYBR Green supermix, 0.8 μM forward and reverse primer, and cDNA in a total volume of 20 μl. The following program was used for denaturation and amplification of the cDNA: 30 sec at 95 °C, followed by 40 cycles of 5 sec at 95 °C and 30 sec at 60 °C. The expression levels of target genes were normalized using GAPDH as an internal control.

Results and discussion

To determine the effect of TBBPA on hepatic lipid metabolism, we measured serum lipid levels. Fig. 1 shows an elevation in serum total cholesterol level in TBBPA treated mice as compared to control mice. HDL- and LDL-cholesterol were also increased. In contrast, TBBPA treatment did not influence for the serum level of triglycerides. Similarly, a difference was not observed in the body weight, food intake and liver weight, comparing to control data of mice. As shown in Fig. 2, when we investigated hepatic lipid content, the levels of hepatic total and LDL-cholesterol were slightly increased. However, the levels of triglycerides and HDL-cholesterol were not affected without TBBPA-treatment.

Liver is a key organ involved in lipid metabolism, as it controls synthesis of many nutrients including lipid and carbohydrates. To evaluate whether cholesterol level alterations in blood and liver were related to changes gene expression like lipid metabolism and PPARγ activity, we investigated hepatic change in expression of several metabolic genes regulated by PPARγ activity. Hepatic mRNA levels of CD36 and fat-specific protein 27 (FSP27) were significantly increased, observing the accumulation of cholesterol in liver of mice. Moreover, mRNA levels of prolipogenic enzymes such as fatty acid synthase (FAS) was also slightly up-regulate.

Many studies have been investigated the neurotoxicity of TBBPA and hormone receptors activity *in vitro*. However, there are only few reports with lipid metabolic effects of TBBPA exposure *in vivo* until present. In this study, these results suggest that TBBPA exposure has effects on total cholesterol levels in blood and liver by promoting expression of lipogenic genes through PPARγ activity. However, the tested dose was fairly high concentration and the tested duration was also short. Further research is needed to investigate the mechanism of up-regulate cholesterol levels in detail and to determine the toxicity of long-term TBBPA exposure.

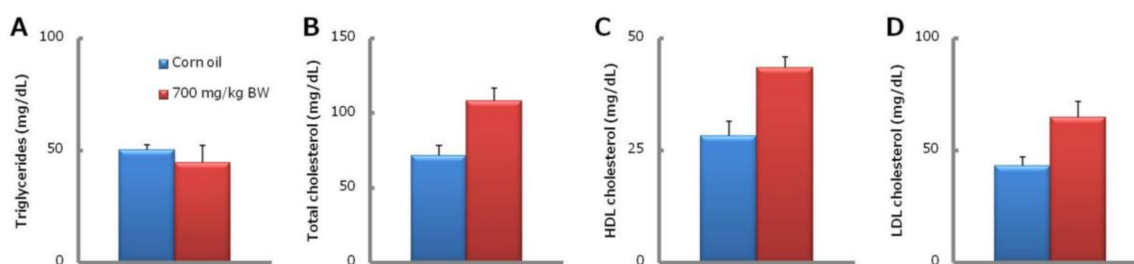

Fig. 1 Effect of TBBPA on serum triglyceride and cholesterol concentrations

(A) Serum triglyceride concentration. (B) Total serum cholesterol concentration. (C) HDL-cholesterol concentration. (D) LDL-cholesterol concentration.Results are means ± SEM (Corn oil: n=6, 700 mg/kg body weight TBBPA: n=5).

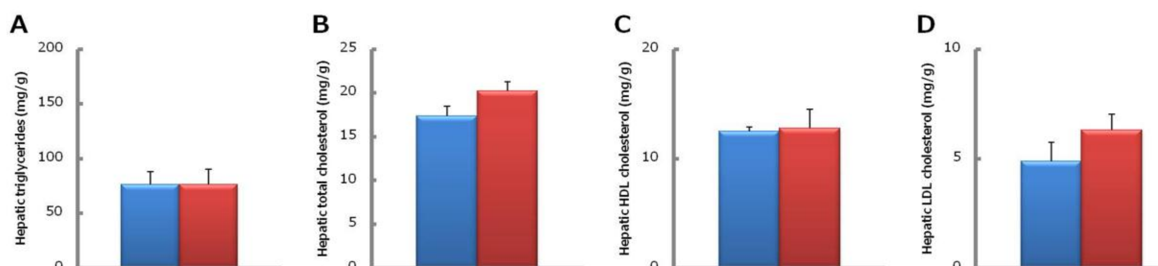

Fig. 2 Effect of TBBPA on triglyceride and cholesterol concentrations in liver

(A) Hepatic triglyceride concentration. (B) Hepatic total cholesterol concentration. (C) HDL-cholesterol concentration. (D) LDL-cholesterol concentration. Results are means ± SEM (Corn oil: n=6, 700 mg/kg body weight TBBPA: n=5).

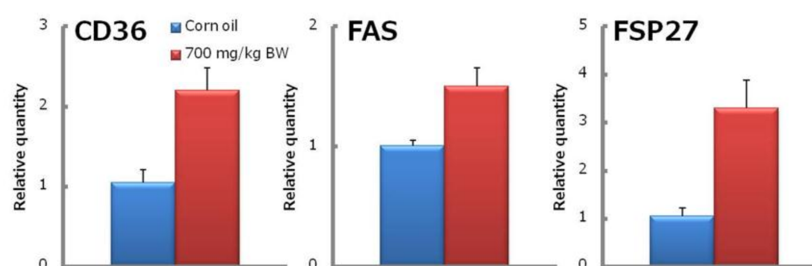

Fig. 3 Effect of TBBPA on lipogenic gene expression in liver

Relative mRNA levels in liver (Corn oil: n=6, 700 mg/kg body weight TBBPA: n=5). Results are means ± SEM.

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