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Tributyltin exposure induces gut microbiome dysbiosis with increased body weight gain and dyslipidemia in mice

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ABSTRACT

Gut microbiome dysbiosis plays a profound role in the pathogenesis of obesity and tributyltin (TBT) has been found as an environmental obesogen. However, whether TBT could disturb gut microbiome and the relationship between obesity induced by TBT exposure and alteration in gut microbiota are still unknown. In order to assess the association between them, mice were exposed to TBTCl (50 μg kg^{-1}) once every three days from postnatal days (PNDs) 24 to 54. The results demonstrated that TBT exposure resulted in increased body weight gain, lager visceral fat accumulation and dyslipidemia in male mice on PND 84. Correspondingly, 16S rRNA gene sequencing revealed that TBT treatment decreased gut microbial species and perturbed the microbiome composition in mice. Furthermore, Pearson's corelation coefficient analysis showed a significantly negative correlation between the body weight and the alpha diversity of gut microbiome. These results suggested that TBT exposure could induce gut microbiome dysbiosis in mice, which might contribute to the obesity pathogenesis.

1. Introduction

There are a vast ensemble of microbes inhabiting in human gut which contain approximately 10^{14} cells and over 100 times more genes than human genome (Ley et al., 2006a; Qin et al., 2010). The gut microbiota provide us with important metabolic capabilities (Turnbaugh et al., 2007) by involving in food digestion, absorption, metabolism as well as other physiological processes (Weaver, 2015). Although it can remain relatively stable once formed after birth (Collado et al., 2012), mounting evidences suggested that gut microbiome could be affected by diet (Walker et al., 2014), antibiotics abuse (Korpela et al., 2016; Ladirat et al., 2013) and chemicals exposure (Ba et al., 2017; Hu et al., 2016; Lai et al., 2016; Zhang et al., 2015).

Nowadays, obesity has become a colossal menace to public health worldwide (Fincham, 2011). It is quite clear that gene background, dietary habit and physical exercise play important roles in bodyweight regulation, meanwhile more and more studies revealed that variation in gut microbiota was associated with obesity (Backhed et al., 2004; Le Chatelier et al., 2013; Ley et al., 2006b; Ojeda et al., 2016). Moreover, obesity phenotype can be transmitted among different individuals via microbiota inoculation in mammals (Ridaura et al., 2013). And further demonstration in a clinical intervention stated that transferring gut microbiota from thin healthy participants could improve insulin sensitivity of diabetes patients (Vrieze et al., 2012). Alteration of the gut microbiome is getting growing attentions in the exploration of pathophysiology and treatment of obesity related disease.

Tributyltin (TBT), a typical endocrine disrupting chemical (EDC), is widely used in agriculture and industry, whose bioaccumulation in aquatic products has posed the most exposure risk to humans (Fang et al., 2017). TBT was identified as an environmental obesogen that can perturb the normal developmental and homeostatic controls over adipogenesis and energy balance (Grun and Blumberg, 2006). Previous studies have shown that TBT could augment adipocyte differentiation *in vitro* (Regnier et al., 2015; Yanik et al., 2011), promote fat accumulation (Grun and Blumberg, 2006) and increase body weight following exposure *in vivo* (Si et al., 2012; Zuo et al., 2011). However, whether the gut microbiome dysbiosis accounts for the obesity phenotype induced by TBT exposure remains unclear.

In the present study, we investigated the impact of TBT exposure on the gut microbiome composition using 16S ribosomal RNA gene (rRNA) sequencing, and elaborated the association between the obesity phenotype and the gut microbiota alteration in mice based on our previous study (Si et al., 2012).

Abbreviations: TBT, tributyltin; EDC, endocrine disrupting chemical; rRNA, ribosomal RNA; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; PND, postnatal day; OUT, operational taxonomic unit; RDP, ribosomal database Project; PcoA, principal coordinate analysis; PPARγ, proliferator activated receptor γ; MSC, mesenchymal stromal stem cell; HFD, high-fat diet; HSD, high-sucrose diet

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2. Materials and methods

2.1. Reagents

TBTCl (purity ≥ 96%) was purchased from Sigma-Aldrich Chemicals Co. (St Louis, MO). Kits for serum triglycerides (TG), total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol were purchased from MedicalSystem Biotechnology Co., Ltd (Ningbo, China). All other chemicals were of analytical grade and were obtained from commercial sources.

2.2. Animals and treatment

21-day-old male SPF ICR mice (10.5–12.5 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd [License No. SCXK (Jing) 20160011]. These mice were fed with temperature (23 °C ± 2 °C) and humidity (55% ± 15%). The animals housed in plastic cages lined with sawdust were kept under a 12-h light-dark cycle. Commercial animal feed and drinking water were available ad libitum. All treatments and experiments were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and followed the principles "Use of Animals in Toxicology", which were supervised by the Ethics Committees of School of Public Health, Shandong University.

After 3 days for acclimatization, the animals were randomly allocated into 2 groups (n = 10 per group), and received TBTCl (50 μg kg^{-1}) or the corn oil (i.p., 5 ml/kg body weight) once every three days, from postnatal days (PNDs) 24 to 54. The mice were sacrificed on PND 84. Four mice were selected randomly in each group to sterilely collect their faeces before sacrifice.

2.3. Serum isolation

Mice were lightly anesthetized and the blood was obtained from the retro-orbital plexus on PND 84. Serum was separated and stored at −80 °C for the measurement of serum analyzation. Serum levels of TG, TC and LDL were checked by an autoanalyser (Beckman Coulter AU480).

2.4. 16S rRNA sequencing library preparation

Bacterial DNA from fecal samples was extracted using the TIANamp Stool DNA Kit (TianGen, China) according to the manufacturer's instructions. DNA concentration and sample integrity testing were performed by agarose gel electrophoresis. To construct the DNA library of 16S rRNA, corresponding primers (seen in Table A.1) and PCR master mix (KAPA HiFi Hotstart PCR Kit, USA) were used to run the PCR. The following PCR conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 25 cycles consisting of denaturation (95 °C for 30 s), annealing (53 °C or 55 °C for 30 s) and extension (72 °C for 30 s), as well a final extension step at 72 °C for 5 min. PCR products were purified with an Ampure Xp beads purification kit (Beckman Coulter, Inc., USA). Purified products were subjected to a further amplification as the method mentioned above. The products were separated by agarose gel electrophoresis, and the target DNA fragments were recovered by a QIAquick Gel Extraction Kit (Qiagen, USA) according to the manufacturer's instructions. Before sequencing, the quality of DNA libraries was assessed by a Qubit Fluorometer (Thermo Fisher Scientific, USA), the length distribution of DNA libraries was assessed by a Qseq100 DNA Analyzer (BiOptic Inc, Taiwan), and the molarity of the DNA libraries was quantified using KAPA Library Quantifcation Kit (KAPA Biosystems, USA). The PCR products were stored at −20 °C for sequencing. The qualified libraries were sequenced by Beijing Ori-gene Science and Technology LTD (Beijing, China) using Illumina MiSeq platform (Paired-end; insert size, 350 bp; read length, 100 bp). And then the image data was converted to original sequencing sequence (raw data) through base calling. Technology Roadmap was shown in Fig. A.1a.

2.5. Sequencing data analysis

Paired-end sequences were merged to tags by FLASH (Fast Length Adjustment of Short reads, v1.2.11) (Magoc and Salzberg, 2011). And then, the raw data was filtered to eliminate the low quality reads, primer sequence and sequence whose length were less than 200 bp or more than 500 bp by Muthor (Schloss et al., 2009), and exclude chimera via UCHIME (Edgar et al., 2011) to obtain clean reads. The clean sequences were clustered into operational taxonomic units (OTUs) based on sequence similarity, whose threshold was 97%. During this process, singletons were abandoned. OTU representative sequences were taxonomically classified using Ribosomal Database Project (RDP) (Wang et al., 2007) trained on SILVA database (Pruesse et al., 2007) as the training sequences. Alpha diversity (within-sample diversity) including community richness, community diversity and community evenness was estimated by Muther. Beta diversity (between-sample diversity) was estimated by UniFrac (Lozupone and Knight, 2005) to generate the inter-community distance matrix, and FastTree software (Price et al., 2009) to constructe a phylogenetic tree. And then the unweighted inter-community distance matrix was used to carry out cluster heat map analysis and principal coordinate analysis (PcoA). The process in this section was shown in Fig. A.1b.

2.6. Statistics

Statistical analyses were conducted using IBM SPSS 20 System (SPSS INC., USA) or R software (MathSoft, USA). Statistical significance of differences between two groups was determined by Student's t test. Differences in the abundance of species between groups were taken via Metastats test. The correlation between the gut microflora and body-weight was generated using Pearson's corelation coefficient. *P* values less than 0.05 were considered to be significant.

3. Results

3.1. TBT exposure increases weight gain and visceral fat accumulation in mice

A significant increase in body weight of mice was observed in the 50 μg kg^{-1} TBTCl group at the end of experiment [42.36 ± 1.97 g for the TBTCl group, and 39.34 ± 1.69 g for the control, $p < 0.01$]. The gain of body weight showed a pronounced difference between these groups as well [18.16 ± 0.52 g for the TBTCl group, and 15.45 ± 0.45 g for the control, $p < 0.01$]. To examine whether this weight gain was due to expansion of adipose tissue, we weighed the epididymal and perirenal adipose tissue. As illustrated in Fig. 1, the mass of visceral fat was significantly larger in TBTCl mice than in control ones ($p < 0.01$).

3.2. TBT exposure results in dyslipidemia in mice

As shown in Table 1, the levels of fasting serum TC, TG and LDL increased significantly in the animals treated with TBT compared to the controls. It is noteworthy that serum LDL level elevated by 2.08 fold in TBT exposure mice.

3.3. Sequencing summary

A total of 455,770 clean sequencing reads were obtained, composed of 210,483 from control and 245,287 from TBT group. Distribution of sequence lengths over all sequences was between 400 bp and 430 bp, as shown in Fig. A.2. Based on similar 16S rRNA sequences, the OTU was used to classify groups of closely related individuals. In our research,

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