Environmental Pollution 226 (2017) 268–276

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Organochloride pesticides modulated gut microbiota and influenced bile acid metabolism in mice☆

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ARTICLE INFO

Article history:
Received 7 January 2017
Received in revised form
22 March 2017
Accepted 26 March 2017
Available online 6 April 2017

Keywords:
Organochloride pesticides
Bile acids metabolism
Intestinal microbiota

ABSTRACT

Organochlorine pesticides (OCPs) can persistently accumulate in body and threaten human health. Bile acids and intestinal microbial metabolism have emerged as important signaling molecules in the host. However, knowledge on which intestinal microbiota and bile acids are modified by OCPs remains unclear. In this study, adult male C57BL/6 mice were exposed to p, p'-dichlorodiphenyldichloroethylene (p, p'-DDE) and β-hexachlorocyclohexane (β-HCH) for 8 weeks. The relative abundance and composition of various bacterial species were analyzed by 16S rRNA gene sequencing. Bile acid composition was analyzed by metabolomic analysis using UPLC-MS. The expression of genes involved in hepatic and enteric bile acids metabolism was measured by real-time PCR. Expression of genes in bile acids synthesis and transportation were measured in HepG2 cells incubated with p, p'-DDE and β-HCH. Our findings showed OCPs changed relative abundance and composition of intestinal microbiota, especially in enhanced *Lactobacillus* with bile salt hydrolase (BSH) activity. OCPs affected bile acid composition, enhanced hydrophobicity, decreased expression of genes on bile acid reabsorption in the terminal ileum and compensatory increased expression of genes on synthesis of bile acids in the liver. We demonstrated that chronic exposure of OCPs could impair intestinal microbiota; as a result, hepatic and enteric bile acid profiles and metabolism were influenced. The findings in this study draw our attention to the hazards of chronic OCPs exposure in modulating bile acid metabolism that might cause metabolic disorders and their potential to cause related diseases in human.

1. Introduction

Bile acids (BAs), the end product of cholesterol metabolism, are synthesized in hepatocytes, secreted into bile and stored in the gallbladder. During meal, they are released into the intestine and are essential for solubilization, absorption, and metabolism of triglycerides, cholesterol, and fat-soluble vitamins (Fiorucci and Distrutti, 2015; Joyce and Gahan, 2016; Klaassen and Cui, 2015). BAs produced in liver are referred to as primary BAs, i.e. cholic acid (CA) and chenodexycholic acid (CDCA). In mice, α-muricholic acid

Abbreviations: OCPs, Organochlorine pesticides; p p'-DDE, p, p'-dichlorodiphenyldichloroethylene; β-HCH, β-hexachlorocyclohexane; BSH, Bile salt hydrolase; BAs, Bile acids; CA, Cholic acid; CDCA, Chenodexycholic acid; αMCA, α-muricholic acid; βMCA, β-muricholic acid; DCA, Deoxycholic acid; LCA, Lithocholic acid; SI, Small intestine; TCA, Taurocholic acid; GCA, Glycocholic acid; TDCA, Taurodeoxycholic acid; GDCA, Glycodeoxycholic acid; TLCA, Taurolithocholic acid; GLCA, Glycolithocholic acid; OTUs, Operational taxonomic units; *Cyp7a1*, Cholesterol 7α-hydroxylase; *Cyp8b1*, Sterol 12α-hydroxylase; *Cyp27a1*, Sterol 27-hydroxylase; *Mrp2/3*, Multidrug resistance-associated protein 2/3; *Abcb11*, ATP-binding cassette, sub-family B, member 11; *Ntcp*, Na^+-taurocholate cotransporting peptide; *Asbt*, Apical sodium bile acid transporter; *Ostα* and *Ostβ*, Organic solute transporters; *Ibabp*, Ileal bile acid binding protein; *Fgf15*, Fibroblast growth factor 15.

☆ This paper has been recommended for acceptance by Dr. Harmon Sarah Michele.

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http://dx.doi.org/10.1016/j.envpol.2017.03.068
0269-7491/

(αMCA) and β-muricholic acid (βMCA) are the major primary BAs. The primary BAs are conjugated to either glycine mainly in humans or taurine in rodents (Liu et al., 2015).

In the colon, bacterial enzymes containing bile salt hydrolase (BSH) activity can deconjugate BAs which are further metabolized through 7α-dehydroxylation to produce more hydrophobic, secondary BAs (Wang et al., 2002). Deoxycholic acid (DCA) and lithocholic acid (LCA) are the two major secondary BAs. Despite trillions of bacteria in the gut, only a few species, such as *Clostridium scindens* and *Lactobacillus*, have been confirmed to have ability to metabolize primary BAs (Ridlon and Hylemon, 2012). Therefore, the composition of the intestine microbiota is a key determinant influencing the composition of BAs (Joyce et al., 2014). It has recently been indicated that, the intestinal microbiota has a vital role in regulating host metabolism by altering bile acid composition (Fiorucci and Distrutti, 2015). Intestinal bacteria live in communities defined by their environment (De Vadder et al., 2014). When the intestinal micro-environment was disrupted by exogenous chemicals, such as antibiotics, abnormal intestinal microbiota could affect many aspects of host physiology (Xu et al., 2013).

Organochlorine pesticides (OCPs) are one kind of banned pesticides in the 1970s and 1980s, but are still widely detected in the environment over the world and human body in recent years (Maisano et al., 2016). p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) and β-hexachlorocyclohexane (β-HCH) are the major breakdown products metabolized from dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH), which are the most well-known among OCPs (Salihovic et al., 2016). OCPs have been affirmed for adverse health effects such as reproductive interference (Freire et al., 2011), cancer (Freund et al., 2014), neurobehavioral disorders (Saeedi Saravi and Dehpour, 2016), immunological toxicity (Mrema et al., 2013) and metabolic disease (Ji et al., 2016; Rantakokko et al., 2015).

However, whether OCPs lead to disorders in modulation of intestinal microbiota or bile acid metabolism has not been ever reported and remains unknown yet. In the present study, mice were exposed to p, p'-DDE and β-HCH at a low dose for 8 weeks which mimics the chronic exposure in human. The influences on intestinal microbiota, bile acid profile and bile acid metabolism by p, p'-DDE and β-HCH were investigated.

2. Materials and methods

2.1. Chemicals

p, p'-DDE and β-HCH were purchased from Tokyo Chemical Industry (Shanghai, China, B0133-1 g, purity>99.0%, CAS No. 72-55-9) and Aladdin (Shanghai, China, H114177-100 mg, CAS No. 319-85-7), respectively.

2.2. Animals and treatments

Male adult C57BL/6 mice (from Shanghai SLAC Laboratory Animal Co., Ltd. Shanghai, China) were fed with standard chow diet. p, p'-DDE (1 mg/kg body weight/day, DDE group), β-HCH (10 mg/kg body weight/day, HCH group) or vehicle (control group) were administered by oral gavage once a day for 8 weeks (n = 8/group). On the day of sacrifice, key organs, such as liver, gallbladder, small intestine (SI), and feces at the distal colon were harvested. Small intestine was equally divided into three parts designated as proximal, middle and distal SI. All experiment protocol was approved by the local Ethical Committee of Nanjing Medical University.

2.3. Bacterial DNA extraction and PCR amplification

Total genomic bacterial DNA was extracted from faecal samples using an E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. PCR amplification of bacteria DNA in the present study were performed as our previous described (Xu et al., 2014; Yan et al., 2015). PCR reactions were performed in triplicate using 20 μL of mixture containing.

2.4. Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gel, purified by the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, Calif., USA), and quantified by QuantiFluorTM-ST (Promega, Madison, Wisc., USA) according to the manufacturer's protocols. Then purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq platform following standard instructions.

2.5. UPLC-MS analysis of bile acid profiles in gallbladder

Ten microliter of gallbladder bile were diluted 100 times with the internal standard in ethanol, vortexed and purified by the sedimentation plate. Then, 100 μL samples were loaded into a centrifuge tube. After lyophilizing, the samples were dissolved with 100 μL 25% acetonitrile, and then centrifuged supernatants were preparing for injection to measure the levels of various bile acids.

Bile acids profiles were analyzed on an Acquity UPLC system coupled to a Waters Xevo TQ-S MS (Waters, Manchester, UK), equipped with a 2.1 × 100 mm C18 reverse-phase column with 1.7 μm particle size (Waters Corp., Milford, MA, USA). Analytes were detected by electrospray ionization and quantified by internal standard methods. The main parameters: nebulizing gas flow 3 L/min, heating gas flow 10 L/min, interface temperature 300 °C, DL temperature 250 °C, heat block temperature 400 °C, drying gas flow 10 L/min. Hydrophobicity index was calculated as described (Heuman, 1989).

2.6. Determination of mRNA expression of genes in bile acid metabolism by quantitative real-time PCR

Total RNA was isolated from liver tissues, distal SI, and HepG2 cells using TRIzol (Invitrogen, Calsbad, CA) according to the manufacturer's protocol. cDNA was synthesized with PrimeScript™ RT Master Mix (Takara, Dalian, China). SYBR Green Master Mix buffer was used for quantitative real-time PCR at final reaction volumes of 10 mL in ABI 7900 HT fast real-time system (Applied Biosystems, Foster City, CA, USA). The specificity of PCR product was validated using performance of a melting curve for each primer set. The reactions were analyzed with the ΔΔCt analysis method to describe mRNA relative expression. RNA data were normalized to GAPDH mRNA. The primer sequences are listed in Supplementary Table 1.

HepG2 cells were cultured with p, p'-DDE (0, 1, 10 ng/mL) and β-HCH (0, 10, 100 ng/mL). Cell viability was determined by cell counting Kit-8 (CCK-8) assay (Beyotime, China).

2.7. Statistical analyses

All values are expressed as mean ± SEM. Multiple groups were evaluated with one-way ANOVA followed by Bonferoni or Dunnett's multiple comparison test, performed with SPSS (version 20.0). All experiments were executed in triplicates and comparisons resulting with P value < 0.05 were considered as statistically significant.