Environmental Pollution 237 (2018) 377–387

Contents lists available at ScienceDirect

Environmental Pollution

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

Dietary grape seed proanthocyanidin extract regulates metabolic disturbance in rat liver exposed to lead associated with PPARα signaling pathway☆

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ARTICLE INFO

Article history:
Received 24 October 2017
Received in revised form
11 February 2018
Accepted 11 February 2018

Keywords:
Hepatic fatty acids metabolism
Grape seed proanthocyanidin extract
Lead
Transcriptional regulation
PPARα signaling pathway

ABSTRACT

Lead, a pervasive environmental hazard worldwide, causes a wide range of physiological and biochemical destruction, including metabolic dysfunction. Grape seed proanthocyanidin extract (GSPE) is a natural production with potential metabolic regulation in liver. This study was performed to investigate the protective role of GSPE against lead-induced metabolic dysfunction in liver and elucidate the potential molecular mechanism of this event. Wistar rats received GSPE (200 mg/kg) daily with or without lead acetate (PbA, 0.5 g/L) exposure for 56 d. According to biochemical and histopathologic analysis, GSPE attenuated lead-induced metabolic dysfunction, oxidative stress, and liver dysfunction. Liver gene expression profiling was assessed by RNA sequencing and validated by qRT-PCR. Expression of some genes in peroxisome proliferator-activated receptor alpha (PPARα) signaling pathway was significantly suppressed in PbA group and revived in PbA + GSPE group, which was manifested by Gene Ontology analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis and validated by western blot analysis. This study supports that dietary GSPE ameliorates lead-induced fatty acids metabolic disturbance in rat liver associated with PPARα signaling pathway, and suggests that dietary GSPE may be a protector against lead-induced metabolic dysfunction and liver injury, providing a novel therapy to protect liver against lead exposure.

1. Introduction

Lead, commonly applied in industrial production for its specific physics features (Bojdi et al., 2014; Zhao and Zhu, 2016), presents as a critical environmental toxicant. It has been clear that lead leached from water system is profoundly dangerous to human health (Gostin, 2016). Approximately, 674,000 deaths annually which would increase continuously, attribute to lead exposure worldwide till 2010 (Lim et al., 2012). It must be recognized that lead poisoning has become one of the pervasive and well-established environmental hazards around the globe.

Lead exposure contributes to metabolic disorder in various tissues (Berglund et al., 2010; Douglas-Stroebel et al., 2004; Jung et al., 2017; Nie et al., 2017; Rogers et al., 2016; Ruiz et al., 2016). However, the mechanisms associated with transcriptional regulation of metabolic dysfunction induced by toxicants are fewer understood

Abbreviations: GSPE, Grape seed proanthocyanidin extract; PbA, Lead acetate; PPARα, Peroxisome proliferator-activated receptor alpha; ROS, Reactive oxidative species; PBS, Phosphate-buffered saline; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TG, Triglyceride; T-CHO, Total cholesterol; GSH, Glutathione; MDA, Malondialdehyde; SOD, Superoxide dismutase; GO analysis, Gene Ontology analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; DE, Differently expressed; FABP1, Fatty acids binding protein 1; SCD1, Stearoyl-CoA desaturase-1; EHHADH, Enoyl-CoA hydratase 3-hydroxyacyl-CoA dehydrogenase; RXR, Retinoid X receptor; iNOS, Inducible nitric oxide synthase; RNS, Reactive nitric species.

☆ This paper has been recommended for acceptance by David Carpenter.

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https://doi.org/10.1016/j.envpol.2018.02.035
0269-7491/

than those of tumorigenesis, obesity, and diabetes interpreted by transcriptome sequencing (Maher et al., 2009; Mani et al., 2016; Glastonbury et al., 2016; Tarifeño-Saldivia et al., 2017). No unbiased deep sequencing analysis of all expressed genes in lead-exposed rat liver has been reported to data. Moreover, efficient treatments of lead exposure with low side effects and metabolic regulation still lack further study.

Natural products has been of great attention in preventing and recovering diseases induced by environmental toxicants (Crescenti et al., 2015; Gil-Cardoso et al., 2017; Tan et al., 2018; Yu et al., 2013; Zhang et al., 2013; Yang et al., 2016; Pan et al., 2017). A potent antioxidant collected from grape seeds named grape seed proanthocyanidin extract (GSPE) provides a rich source of polyphenols. As a metabolic regulator and reactive oxidative species (ROS) scavenger, GSPE plays a crucial role in antioxidation, antiinflammation, radical scavenging, and metabolic regulation (Crescenti et al., 2015; Gil-Cardoso et al., 2017). In addition, free flavanols and their phase II metabolites (catechin, epicatechin, procyanidin dimers, gallic acid and their methylated, glucuronidated and sulfated derivatives) reached the concentration at 200 nmol/g in rat liver under an administered dose at 250 mg/kg of GSPE (Margalef et al., 2015), suggesting that bioactivity of GSPE can target liver tissue. Thus, GSPE may potentially play a protective role in resisting against chronic lead exposure-induced metabolic dysfunction in rat liver.

Compared to the previous studies about the protective effect of GSPE against lead (Liu et al., 2014, 2017, 2018; Long et al., 2016; Zhang et al., 2004), we firstly hypothesized that GSPE would ameliorate metabolic disorder in rat livers induced by chronic lead poisoning. In the experiment, we used RNA sequencing to investigate changes in genes expression altered by lead and GSPE. This study, for the first time, focuses on the molecular mechanism by which GSPE ameliorates lead-induced metabolic disorder, and identifies potential signaling pathway by RNA sequencing to increase knowledge of the molecular bases of metabolic disorder caused by lead and the protective effect of dietary GSPE.

2. Materials and methods

2.1. Animals and treatments

Healthy male Wistar rats (120 ± 20 g body weight, 6–8 weeks age, n = 40) were obtained from Experimental Animal Centre of Harbin Medical University (Harbin, China) and acclimated for a week before the start of experiment, under the same laboratory condition with consume food and water ad libitum. Before and during the experimental period, rats were housed under environmental conditions with a 12 h interval light/dark cycle, a minimum of 40% relative humidity, and a room temperature of 21 ± 4 °C, and were provided with standard pelleted rodent diet.

Rats were randomly divided into 4 groups: control, PbA, PbA + GSPE, and GSPE. All treatments lasted 56 d. In the PbA group, drinking water was suspended with PbA (0.5 g/L). In the PbA + GSPE group, rats were orally given GSPE (200 mg/kg) daily with the presence of PbA in drinking water. In the GSPE group, rats were orally given GSPE (200 mg/kg) without PbA. All rats were fed with NTP-2000 diet (Trophic Animal Feed High-Tech Co., Ltd, Nantong, China). The animal protocol was approved by the Ethical Committee for Animal Experiments (Northeast Agricultural University, Harbin, China). Ether anesthesia were given 24 h after the last treatment. Blood samples from the abdominal vein were taken with vacuum tubes containing heparin sodium anticoagulant. Liver tissues were rapidly collected and homogenized in phosphate-buffered saline (PBS) (pH 7.4, w/v; 1 g tissue with 9 mL PBS) with an Ultra-Turrax T25 Homogenizer. After centrifugation at 10000 × g for 10 min at 4 °C, the supernatant was applied for biochemical analysis.

2.2. Biochemical analysis

The blood samples were centrifuged at 3000 × g for 10 min. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and concentration of total triglyceride (TG) and total cholesterol (T-CHO) in serum were detected with a Uni Cel DxC Synchron chemistry system (Beckman Coulter Inc., Fulton, CA, USA).

2.3. Analysis of liver GSH and MDA concentration and SOD activity

Liver glutathione (GSH) and malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were examined according to manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Histopathology

Liver tissues from rats fixed in 4% formaldehyde overnight at 4 °C and then cut into blocks of 3 mm thickness. After embedded in paraffin, sections (5 μm thickness) were cut on the coronal plane and stained with hematoxylin and eosin. Morphology was examined by a light microscope (BX-FM: Olympus Corp, Tokyo, Japan).

2.5. RNA extraction and purification

Total RNA from liver samples was extracted using RNAiso Plus Total RNA extraction reagent (Cat #9109, TAKARA Bio Inc., Otsu, Shiga, Japan) according to the manufacturer's instructions and inspected for a RIN number to examine RNA integrity by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). Further purification of qualified total RNA was performed by RNAClean XP Kit (Cat A63987, Beckman Coulter Inc., Brea, CA, USA) and RNase-Free DNase Set (Cat #79254, QIAGEN GmbH, Duesseldorf, Germany).

2.6. RNA sequencing

Samples of RNA isolated from liver tissues with integrity values > 7 were used for library preparation. Then using the TruSeq RNA Sample Preparation Kit (RS-122-2001, Illumina, San Francisco, CA, USA) were used to synthesize the paired-end libraries following the supplied guidelines. Library construction and Illumina sequencing were achieved by the Shanghai Biotechnology Corporation. High-quality reads for sequence analysis and bioinformatic data analysis were also supplied by the Shanghai Biotechnology Corporation.

2.7. RNA sequencing mapping and bioinformatic analysis of mRNA data

Before read mapping, clean reads were achieved from the raw reads by filtering out rRNA reads, sequencing adapters, short-fragment reads and other low-quality reads. Clean reads were aligned to the rat genome (ensembl rn6) by Tophat v2.0.9 (Trapnell et al., 2009). The differentially expressed genes (DE genes) were identified by EBSeq algorithms. Genes were considered significantly differentially expressed under the following criteria: (1) fold change > 2, and (2) adjusted FDR (q value) < 0.05 (Reiner et al., 2003; Turcan et al., 2012).

To identify potential functions of lead and GSPE, RNA sequencing analysis of DE genes was performed using Gene