Toxicology and Applied Pharmacology 318 (2017) 41–48

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Perfluorooctanoic acid affects endocytosis involving clathrin light chain A and microRNA-133b-3p in mouse testes

Yin Lu [a,1], Jianshe Wang [a,1], Xuejiang Guo [b], Shengmin Yan [a], Jiayin Dai [a,*]

[a] Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, PR China
[b] State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing 210029, PR China.

ARTICLE INFO

Article history:
Received 2 November 2016
Revised 19 January 2017
Accepted 22 January 2017
Available online 24 January 2017

Keywords:
Perfluorooctanoic acid
miRNA
Endocytosis
CLTA

ABSTRACT

Perfluorooctanoic acid (PFOA) is an abundant perfluoroalkyl substance widely applied in industrial and consumer products. Among its potential health hazards, testicular toxicity is of major concern. To explore the potential effect of miRNA on post-translational regulation after PFOA exposure, changes in miRNAs were detected *via* miRNA array. Seventeen miRNAs were differentially expressed (eight upregulated, nine downregulated) in male mouse testes after exposure to 5 mg/kg/d of PFOA for 28 d (>1.5-fold and $P < 0.05$ compared with the control). Eight of these miRNAs were further selected for TaqMan qPCR analysis. Proteomic profile analysis indicated that many changed proteins after PFOA treatment, including intersectin 1 (ITSN1), serine protease inhibitor A3K (Serpina3k), and apolipoprotein a1 (APOA1), were involved in endocytosis and blood-testis barrier (BTB) processes. These changes were further verified by immunohistochemical and Western blot analyses. Endocytosis-related genes were selected for qPCR analysis, with many found to be significantly changed after PFOA treatment, including epidermal growth factor receptor pathway substrate 8 (Eps8), Eps15, cortactin, cofilin, espin, vinculin, and zyxin. We further predicted the potential interaction between changed miRNAs and proteins, which indicated that miRNAs might play a role in the post-translational regulation of gene expression after PFOA treatment in mouse testes. Among them, miR-133b-3p/clathrin light chain A (CLTA) was selected and verified *in vitro* by transfection and luciferase activity assay. Results showed that PFOA exposure affects endocytosis in mouse testes and that CLTA is a potential target of miR-133b-3p.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Perfluoroalkyl substances (PFASs) are characterized by stable and strong carbon-fluoride bonds with unique water- and oil-repellent properties, resulting in their wide application in industrial and consumer products and environmental persistence (Calafat et al., 2007). Reports have shown that PFASs are not only found in various environmental matrices, but also in serum and tissue samples from human and animal populations (Giesy and Kannan, 2002). Among PFASs, eight-carbon-chain perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two of the most abundant. Studies have shown that PFOA is dose-dependently accumulated in laboratory animal samples, including in serum, liver, and testes (Yan et al., 2014; Zhang et al., 2014; Yan et al., 2015). In addition, PFOA has a relatively long half-life in human serum of 3.8 years (Olsen et al., 2007). Although many countries have restricted the industrial production of PFOA since 2000 (US EPA, U.E.P.A, 2016), its persistence as one of the most dominant PFASs in the environment has remained due to its continued production in countries without legal restriction and *via* degradation of its precursors (Prevedouros et al., 2006; Wang et al., 2009).

Both the environmental persistence and pervasive distribution of PFASs have increased concern in regards to their potential toxicity. Extensive research on the potential health hazards of PFOA exposure has been conducted (Butenhoff et al., 2004; Kennedy et al., 2004; Lau et al., 2004; Lau et al., 2007; Olsen and Zobel, 2007; Olsen et al., 2009). Among these potential health hazards, testicular toxicity is of major concern. Laboratory studies on adult male rats have shown that PFOA reduces testosterone and increases estradiol levels following exposure (Lau et al., 2007). A cross-sectional study reported negative associations between high combined levels of PFOA and PFOS and the proportion of morphologically normal spermatozoa in adult men (Joensen et al., 2009). In addition, luteinizing hormone and free testosterone were found to be positively correlated with plasma PFOA in men attending an *in vitro* fertilization clinic (Raymer et al., 2012).

Abbreviations: UTR, 3′ untranslated region; APOA1, Apolipoprotein a1; BTB, Blood-testis barrier; CLTA, Clathrin light chain A; ITSN1, Intersectin 1; iTRAQ, Isobaric tags for relative and absolute quantitation; miRNA, MicroRNA; PFOA, Perfluorooctanoic acid; PFASs, Perfluoroalkyl substances; Serpina3k, Serine protease inhibitor A3K.

* Corresponding author at: Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, PR China.
E-mail address: daijy@ioz.ac.cn (J. Dai).

[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.taap.2017.01.014
0041-008X/© 2017 Elsevier Inc. All rights reserved.

MicroRNAs (miRNA) are endogenous, short RNA molecules that recognize base-paired complementary sites on their target genes and block target gene translation or trigger degradation of target mRNA (Bartel, 2004). During spermatogenesis, accurately regulated gene expression is fundamental. Animal studies have shown significantly different testicular miRNA profiles between immature and mature animals (Yan et al., 2007; Luo et al., 2010), implying that post-transcriptional control of gene expression by miRNAs participates in spermatogenesis. In addition, among the more than 100 miRNA species found in mammalian testes, about 40% are differentially expressed between testicular and somatic tissues (Ro et al., 2007; Luo et al., 2010). Aberrant miRNA expression has been observed in many diseases (Wahid et al., 2010), suggesting the possibility that environmental pollutants could trigger these changes (Wang et al., 2015). We hypothesized that PFOA can trigger miRNA expression pattern alteration, which might represent a novel mechanism for the testicular toxicity of PFOA. In our previous study, we used isobaric tags for relative and absolute quantitation (iTRAQ) to determine proteomic profile changes in mouse testes after PFOA exposure (Zhang et al., 2014). In the present study, we identified differentially expressed miRNAs in mouse testes after PFOA exposure. Combined with the previous differentially expressed proteins, we explored testicular miRNA alteration and the potential interaction between miRNAs and changed proteins in testes after PFOA treatment to gain insight into the molecular mechanisms of the testicular toxicity of PFOA.

2. Materials and methods

2.1. Animals and treatment

Male BALB/c mice (aged 6–8 weeks) were randomly grouped ($n = 10$ per group) and dosed by oral gavage with Milli-Q water or 1.25, 5, or 20 mg/kg body weight/d of PFOA (Sigma-Aldrich, CAS No. 307-55-1, 99% purity, St. Louis, MO, USA) for 28 d. After treatment, the mice were sacrificed and their testes were either fixed in 4% paraformaldehyde or stored at −80 °C after being immediately frozen in liquid nitrogen. All procedures were approved by the Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences.

2.2. Protein network

Proteomic profile (iTRAQ) analysis was performed on testes from the control and 5 mg/kg/d PFOA-treated animals (Zhang et al., 2014). Proteins with altered expression were selected for further analysis of endocytosis and blood-testis barrier (BTB)-related networks, and this analysis was a secondary analysis based on a previous proteomic profile. All detailed iTRAQ assay data, differentially expressed protein identification, and Pathway Studio analysis *via* the ResNet database (version 6.5, Ingenuity Systems, Inc.) are shown in our previous study (Zhang et al., 2014).

2.3. Western blot analysis

Intersectin 1 (ITSN1), serine protease inhibitor A3K (Serpina3k), and apolipoprotein a1 (APOA1) were analyzed by Western blot assay using testicular protein extracts from the control and 1.25, 5, and 20 mg/kg/d PFOA treatment groups. Protein bands were analyzed with Quantity One software (v 4.6.3, Bio-Rad, USA) and the data were normalized to GAPDH levels.

2.4. qPCR of mRNA

To verify that PFOA treatment altered endocytosis in the mouse testes, genes involved in endocytosis were selected for real-time quantitative PCR analysis. Total RNAs from the testes of the control and the 1.25, 5, and 20 mg/kg/d PFOA treatment groups were isolated using the RNeasy plus Mini Kit (Qiagen, USA). cDNA synthesis and qPCR were performed as per our previous description (Wang et al., 2015), and 18S rRNA was used as the internal control. Primer sequences are listed in Supplementary Table S1. The qPCR data were analyzed with MxPro qPCR software, and the comparative CT ($2^{-\Delta\Delta CT}$) method was used to calculate the fold change of mRNA levels (Livak and Schmittgen, 2001).

2.5. MiRNA array

To be consistent with the doses used in our previous iTRAQ assay (Zhang et al., 2014), animals from the control and 5 mg/kg/d PFOA exposure group were chosen for miRNA array. Testes from two random individuals in the same group were pooled into one sample, and three pooled samples from each group were used for hybridization in the mouse miRNA arrays (Agilent Technologies, Santa Clara, CA, USA). MiRNAs with more than 1.5-fold alteration ($P < 0.05$) were deemed to be significantly changed by PFOA treatment.

2.6. TaqMan qPCR analysis of miRNA

Eight significantly changed miRNAs (miR-133b-3p, miR-365-3p, miR-17-3p, miR-193-5p, miR-191-5p, miR-184-3p, miR-410-3p, and miR-187-3p) from the miRNA analysis were selected for TaqMan qPCR assay (Life Technologies-Applied Biosystems, CA, USA) to confirm the results and detect the miRNA levels in the 1.25, 5, and 20 mg/kg/d PFOA exposure groups. U6 snRNA served as the internal control. All processes were performed according to the manufacturer's protocols, and the comparative CT ($2^{-\Delta\Delta CT}$) method was used to calculate the fold change of miRNA levels (Livak and Schmittgen, 2001).

2.7. miRNA-target network prediction

The networks between altered miRNAs and their potential targets from the differentially expressed proteins were constructed using the online TargetScan database and drawn using Cytoscape (v 3.2.1) software.

2.8. Plasmid transfection and dual-luciferase reporter assay

The 3′ untranslated region (UTR) of CLTA mRNA contained sequences complementary to the seed sites of miR-133b-3p. We amplified this relevant 3′ UTR region from the mouse genomic DNA, as well as its mutated sequence in the putative binding site with miR-133b-3p, and inserted it into psiCHECK-2 dual-luciferase reporter plasmid (Promega, Madison, WI, USA), designated as psi-CLTA 3′ UTR-wt and psi-CLTA 3′ UTR-mut, respectively. Details on the primers used for the above amplification are shown in Supplementary Table S2. The HEK 293T cells were transfected with 1 μg of the above psiCHECK-2 recombinant vector and 50 nM miR-133b-3p agomir (miR-133b-3p) or 50 nM agomir Negative Control (NC) (RiboBio Co., Ltd. Guangzhou, China), and then measured with Synergy™ 2 multi-mode readers 133 (BioTek, Vermont, USA) at 48 h after transfection.

2.9. Immunohistochemical analysis

Testes from the control and three treatment groups were fixed in 4% paraformaldehyde at room temperature (RT) for 1 d, dehydrated in 30% sucrose solution, and then embedded in OCT compound (Sakura Finetek USA, Torrance, CA, USA). Using a Leica CM1900 cryostat (Heidelberger, Nussloch, Germany), frozen sections (5 μm) were sliced, then air dried and fixed in 4% paraformaldehyde at RT for 10 min. The slices were rinsed in PBS-T buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.5% TritonX-100), and then incubated with 3% H_2O_2 in the dark at RT for 15 min to block endogenous peroxidase reaction. After blocking, the slices were incubated with rabbit antibodies of CLTA and peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Delaware Avenue, CA, USA). Brown colored hybrid proteins were

Download English Version:

https://daneshyari.com/en/article/5558624

Download Persian Version:

https://daneshyari.com/article/5558624

Daneshyari.com