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SLC6A19 is a novel putative gene, induced by dioxins via AhR in human hepatoma HepG2 cells☆

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ABSTRACT

The aryl hydrocarbon receptor (AhR) plays an important role in mediating dioxins toxicity. Currently, genes of P450 families are major research interests in studies on AhR-mediated gene alterations caused by dioxins. Genes related to other metabolic pathways or processes may be also responsive to dioxin exposures. Amino acid transporter B0AT1 (encoded by SLC6A19) plays a decisive role in neutral amino acid transport which is present in kidney, intestine and liver. However, effects of dioxins on its expression are still unknown. In the present study, we focused on the effects of dioxin and dioxin-like compounds on SLC6A19 expression in HepG2 cells. We identified SLC6A19 as a novel putative target gene of AhR activation in HepG2 cells. 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) increased the expression of SLC6A19 in time- and concentration-dependent manners. Using AhR antagonist CH223191 and/or siRNA assays, we demonstrated that certain AhR agonists upregulated SLC6A19 expression via AhR, including TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD), 2,3,4,7,8- pentachlorodibenzofuran (2,3,4,7,8-PeCDF) and PCB126. In addition, the expression of B0AT1 was also significantly induced by TCDD in HepG2 cells. Our study suggested that dioxins might affect the transcription and translation of SLC6A19 in HepG2 cells, which might be a novel putative gene to assess dioxins' toxicity in amino acid transport and metabolism in liver.

1. Introduction

Recently the pollution of dioxin and dioxin-like compounds has come to light, in that certainly these chemicals have been shown to be toxic to the environment and to humans. These compounds are almost exclusively produced as industrial products or byproducts which include polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), polybrominated dibenzo-p-dioxins (PBDDs), dibenzofurans (PBDFs), and dioxin-like biphenyls (DL-PCBs). They accumulate in the environment and are enriched through the food chain because of their long half-life (Sinkkonen and Paasivirta, 2000). Thus, they can be widely detected in the environment (e.g., food, water, air, soils and sediments.) and in human (e.g., milk and serum) (Srogi, 2008; Zober et al., 1992). A number of epidemiological and experimental studies have shown that these compounds have diverse adverse health effects (Birnbaum et al., 2003; Greim, 1997; Van den Berg et al., 2006; Taylor et al., 2013; Bertazzi et al., 2001), such as liver toxicity, developmental and reproductive toxicity to immune system dysfunction. These effects have shown to be mediated by activation of aryl hydrocarbon receptor (AhR). The inactivated AhR is usually located in cytoplasm, it enters into nucleus once being activated, which then binds to dioxin-responsive elements (DREs) in the upstream promoter region of a diverse battery of genes and regulates their expression. The gene

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alterations in response to the activation of AhR pathway result in various biological and toxicological effects in different species and tissues (Furness and Whelan, 2009; Marinković et al., 2010).

Liver plays a pivotal role in metabolism of xenobiotic compounds, such as environmental contaminants and drugs. Some of these compounds generate hepatic damage and impair its function. It was well-known that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) could induce pathological changes in mouse and rat liver (Fernandez-Salguero et al., 1996; Gupta et al., 1973). In addition, TCDD upregulates genes encoding enzymes for metabolizing xenobiotics, including cytochrome P450 (CYP) 1A (Zeiger et al., 2001), CYP1B (Xu et al., 2005), and anterior gradient protein 2 homolog (AGR2) (Ambolet-Camoit et al., 2010) in cell lines. Liver is also a significant organ for amino acid transport in the body. Amino acid availability and transport play essential roles in hepatocellular growth, development or regeneration after trauma, necrosis, or other damage (Mailliard et al., 1995). A human hepatoma cell line, HepG2, has been universally applied to detect the toxic effects of hepato-toxins such as apoptosis, necrosis and toxicogenomic effect (Marinković et al., 2010; Fernandez-Salguero et al., 1996; Gupta et al., 1973). Genes associated with amino acid metabolism (e.g. amino acid transporter solute carrier gene families) were found to be regulated by TCDD in HepG2 cells (Frueh et al., 2001; Puga et al., 2000). Jennen et al. (2011) have also shown that amino acid metabolism was widely impacted in HepG2 exposed to TCDD by a cross transcriptomics and metabonomics study. Thus, the transport and metabolic pathway of amino acid are responsive to dioxin exposure in HepG2 cells. However, the related responsive genes are still not fully understood.

B0AT1 is a neutral amino acid transporter encoded by SLC6A19 gene, which plays an important role in transporting neutral amino acids (NAA) and Na^+, involving in protein digestion and absorption, and mineral absorption. It has been reported that mutations in SLC6A19 result in several diseases, such as hartnup disorder (Bröer, 2009), iminoglycinuria (Vanslambrouck et al., 2010) and hyperglycinuria (Bröer et al., 2008). B0AT1 is highly expressed in kidney and intestine, compared to lower expression in other tissues such as pancreas, stomach, liver, duodenum and ileocecum (Kleta et al., 2004). The tissue-specific expression of B0AT1 might be due to transcriptional or epigenetic regulation of SLC6A19 gene (Tümer et al., 2013). However, whether SLC6A19 is one of the responsive genes in dioxin-induced alterations in amino acid transportation in HepG2 cells is still not known. In this study, we elaborated three issues as follow: (1) the effects of dioxin and dioxin-like compounds on SLC6A19 expression; (2) whether these effects were mediated by AhR; (3) whether TCDD affected the expression of B0AT1. Given the function of B0AT1 in amino acid transportation and metabolism, the data of the present study will provide new insight in understanding the molecular basis of the interfering effects of dioxin on the metabolic processes in hepatocytes.

2. Materials and methods

2.1. Chemicals and reagents

TCDD (toxic equivalency factor (TEF) = 1.0), 1,2,3,7,8-PeCDD (PeCDD, TEF = 1.0), 2,3,4,7,8-PeCDF (PeCDF, TEF = 0.3) and PCB126 (TEF = 0.1) were all from Wellington laboratories Inc. (Ontario, Canada). Dimethyl sulfoxide (DMSO) and CH223191 were obtained from Sigma, St. Louis, MO, USA. alpha-MEM and Penicillin-Streptomycin (P/S) were purchased from Invitrogen (Carlsbad, CA, USA).

Fetal bovine serum (FBS) was from Gibco.

2.2. Dioxin and dioxins-like compounds effects on SLC6A19 expression

HepG2 cells was obtained from Dr. Michael S. Denison (University of California, Davis, Davis, CA) as a gift, cultured in alpha-MEM consisted of 10% FBS and 1% P/S and incubated at 37 °C in a water-saturated 5% CO2 incubator. Before exposure to chemical compounds, which were all dissolved in DMSO with the final concentration of 0.1% (v/v), HepG2 cells were seeded in 6-well plates at 300,000 cells/well for 24 h before being exposed to fresh medium containing chemicals. All chemicals were added to HepG2 cells once, and the cells were harvested after different time period of incubations for the following experiments. TCDD was administrated at concentrations of 10^{-10} M or 10^{-8} M for different time periods (2 h, 4 h, 8 h, 24 h, 48 h and 72 h) to examine time-dependent response. TCDD was used at concentrations of 10^{-11} M to 10^{-7} M for 24 h for revealing concentration-dependent response. The effects of other dioxin-like compounds on SLC6A19 expression were also tested, including PeCDD (10^{-9} M), PeCDF (10^{-8} M) and PCB126 (10^{-8} M). Two assays were employed to test the role of AhR, one using an AhR antagonist, CH223191 (Zhao et al., 2010) and the other using siRNA against AhR (siAhR). HepG2 cells were incubated with 10^{-9} M TCDD, 10^{-9} M PeCDD, 10^{-8} M PeCDF or 10^{-8} M PCB126, in company with 10 μM CH223191 for 24 h to see the alteration in SLC6A19 expression. On the other hand, HepG2 cells were firstly transfected with siAhR (10 nM, s1198, Life technologies, USA) using Lipofectamine® LTX Reagent (Invitrogen, CA, USA) in light of the directions for 24 h, and then incubated with DMSO or TCDD (10^{-9} M) for another 24 h to see the effects on SLC6A19 expression.

2.3. Quantitative real-time PCR

Total RNA was extracted from treated cells using TRIzol reagent (Invitrogen), and then genomic DNA was removed with DNaseI (Invitrogen,CA, USA). The quality of RNA was assessed using RNA gel electrophoresis and the quantity was quantified by a NanoDrop 2000 spectrophotometer (NanoDrop, Thermo Scientific). For each sample, 2.5 μg total RNA was used to synthesize cDNA according to the instructions of Moloney Murine Leukemia Virus Reverse Transcriptase kit (Invitrogen, CA, USA). For SLC6A19 and AhR quantitation, cDNA was amplified according to GoTaq® qPCR Master Mix kit protocol (Promega, CA, USA). The specific primers are: GAPDH-forward, 5′-GAGCCACATCGCTCAGAC-3′, GAPDH-reverse, 5′-CTTCTCATGGTTCACACCC-3′; human SLC6A19-forward, 5′-CCCAACATCTTCTGGCAAGT-3′, human SLC6A19–reverse, 5′-GAGGGCACTCCAGCCACAAT-3’; human AhR-forward, 5′-GTGACTTGTACAGCATAATG-3′, human AhR-reverse, 5′-ATCTTCTGACACAGCTGTTG-3′. SLC6A19, AhR and GAPDH cDNAs were amplified using the same reaction mixture, containing 2x PCR Master Mix (Promega, CA, USA), 0.2 μM of primers (each 0.5 μM), and 2 μL cDNA. The PCR program was as following: 1 cycle of 5 min at 95 °C, 45 cycles of 10 s of denaturation at 95 °C, 20 s at 60 °C, and 30 s at 72 °C, and a cooling step of 16 s at 72 °C (ramp rate of 1.5 °C/s). Quantitative real-time PCR was carried out with GoTaq® qPCR Master Mix on a LightCycler 480 Real-time PCR detection system (LC-480II, Roche, USA). GAPDH was served as an internal control for data analysis. The Ct values of GAPDH were slightly changed among different treatment groups. The Ct values of GAPDH were 11.12 ± 0.05 in DMSO solvent control group and 11.36 ± 0.05 in TCDD (10^{-8} M) group which had the most obvious induction effect on SLC6A19 expression (approximately 80-fold induction).