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Effects of acute exposure to polybrominated diphenyl ethers on retinoid signaling in zebrafish larvae

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ABSTRACT

The objectives of the present study were to investigate the effects of acute exposure to PBDEs on retinoid signaling in fish. Zebrafish embryos (2 h post-fertilization, hpf) were exposed to DE-71 (0, 31.0, 68.7, and 227.6 μg/L) until 120 hpf. Retinoid profiles showed the content of retinal and retinoic acid was reduced significantly. While a significant up-regulation was observed in the transcription of retinal dehydrogenase (*raldh2*), the transcription of retinol binding protein (*rbp1a*), retinol dehydrogenase (*rdh1*), cellular retinoic acid binding protein (*crabp1a* and *crabp2a*) and retinoic acid receptor subunit (*raraa*) were down-regulated significantly, indicating disruption of retinoid signaling. However, the transcriptions of five opsin genes (*zfrho*, *zfuv*, *zfred*, *zfblue*, and *zfgr1*) were up-regulated. Furthermore, whole mount immunostaining and western blotting demonstrated increased rhodopsin protein expression in the exposure groups. Overall, the results indicated that acute exposure to PBDEs could disturb retinoid signaling and may impact on eye development of zebrafish larvae.
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1. Introduction

The extensive use of polybrominated diphenyl ethers (PBDEs) as flame retardants has resulted in global contamination and these compounds have been detected in the environment, wildlife and humans (Law et al., 2006). Although concentrations of PBDEs in water samples are generally low, higher concentrations of PBDEs have been detected in fish due to bioaccumulation in aquatic organisms through the food web. For instance, PBDEs were found to be at 2235 ng/g of lipid in San Francisco Bay (Holden et al., 2003), while PBDE concentrations in fish muscles ranged from 35.1 to 1088 ng/g wet weight in an e-waste recycling site in Guiyu, Guangdong, South China (Luo et al., 2007). Notably, high concentrations of PBDEs have been detected in the eggs of Chinese sturgeon (21.2 ng/g wet weight) (Zhang et al., 2010) and striped bass (138.0 ng/g) (Ostrach et al., 2008), indicating maternal transfer of PBDEs to progeny. Moreover, PBDEs concentrations were significantly greater in zebrafish eggs than in the parental female zebrafish after dietary exposure (Nyholm et al., 2008). Thus, fish embryos can be exposed to these compounds during the early stages of embryogenesis.

Research on the toxicity of PBDEs has mainly focused on neurotoxicity and endocrine disruption (Birnbaum and Cohen Hubal, 2006; Costa and Giordano, 2007). However, increasing evidence shows that vitamin A (retinoid) homeostasis is affected in rats, mice and American kestrels after exposure to PBDEs (Ellis-Hutchings et al., 2006; Fernie et al., 2005; Hallgren et al., 2001; van der Ven et al., 2008). Exposure to PBDEs could

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decrease hepatic retinoid storage and interfere with retinoid dynamics.

Retinoid is involved in a wide range of physiological processes, including reproduction, cell growth and differentiation, immunity, embryogenesis, and vision (Zile, 2001). The mobilization of the storage retinyl ester into retinol and its delivery to target tissues is a highly regulated process (Bellovino et al., 2003; Mos et al., 2007). During normal development in fish embryos, retinol binds to the plasma retinol binding protein (RBP) monomer (Lubzens et al., 2003), and then it is transported via the blood from the yolk to various target organs including eyes (Wei, 2003). In the eyes, retinol is transported by cellular retinol binding protein type I (CRBP I). The retinol is catalyzed via a two-step conversion to retinoic acid (RA). Initially, retinol is converted to retinal by retinol dehydrogenase (RDH) in a rate-limiting reaction, and then retinal is converted to the bioactive metabolite RA by retinal dehydrogenase (RALDH), via an irreversible oxidation step (Dobbs-McAuliffe et al., 2004).

RA is an important signaling molecule for photoreceptor development in the visual systems of vertebrates (Prabhudesai et al., 2005). In target cells, after being transported by cellular retinoic acid binding protein (CRABP) into the nucleus, RA binds to RA receptors (RARs) and retinoid X receptors (RXRs), which initiates the transcription of specific genes that contribute to developmental patterning of the hindbrain, spinal cord, heart, and eye (Novák et al., 2008). However, RA in non-target cells is catalyzed to oxidized derivatives by an RA-inducible gene, *cyp26a*, in order to regulate RA activity and avoid RA-induced teratogenesis (Dobbs-McAuliffe et al., 2004; White et al., 1996). Meanwhile, in the eyes, the chromophore retinal binds covalently to photoreceptor opsins to form visual pigments during photoexcitation to initiates the visual transduction cascade. Opsins are synthesized by the photoreceptors and thus determine the spectral identity of the visual pigment (Vihtelic et al., 1999).

Despite the reported susceptibility of retinoid to PBDEs stress and the physiological roles of retinoid in vision, the effects of PBDEs exposure on retinoid transport, metabolism and signaling, and the ultimate effects of these changes on the eye development, remain largely unknown. Therefore, the objectives of the present study were to investigate the effects of exposure to PBDEs on retinoid signaling and the potential impacts on the eye development. For this, zebrafish embryos were exposed to various concentrations of DE-71, a commercial mixture of PBDEs, and retinoid profiles were determined in the larvae using high performance liquid chromatography (HPLC). The transcriptions of genes associated with retinoid transport and metabolism were measured by quantitative RT-PCR. Expressions of opsins were also examined.

2. Materials and methods

2.1. *Chemicals*

DE-71 was obtained from Wellington Laboratories, Inc. (Ontario, Canada; purity > 99.9%), while dimethyl sulfoxide (DMSO) was from Amresco (Solon, OH, USA; purity > 99.9%). Mouse rhodopsin primary antibody was purchased from Abcam (Cambridge, UK). Standards for retinol, retinal and retinoic acid were obtained from Sigma–Aldrich (St Louis, MO, USA). Chemicals used for retinoid measurement were of HPLC grade. Other chemicals used were of analytical grade.

2.2. *Zebrafish maintenance and DE-71 exposure experiments*

Adult zebrafish (AB strain) maintenance and embryos exposure were carried out as detailed by Yu et al. (2010). Embryos that developed normally and reached the blastula stage (2 h post-fertilization, hpf) were selected for further experimentation. The embryos were incubated in glass beakers containing 500 mL nominated DE-71 exposure solution (0, 100, 300 and 1000 μg/L) under a cycle of 14 h light and 10 h darkness at 28 ± 0.5 °C. The selected exposure concentrations were based on a previous study (Lema et al., 2007). Control and treated embryos received 0.001% (v/v) DMSO. During the exposure period, 50% of the solution was renewed daily to ensure appropriate DE-71 concentrations were maintained. Each experimental and control group contained three replicates. At 120 hpf, the larvae were randomly sampled, immediately frozen in liquid nitrogen and stored at −80 °C for subsequent gene, protein and retinoid assays. The larvae were examined under a stereomicroscope for morphological deformities, including pericardial edema and axial spinal curvature.

2.3. *PBDE extraction and analysis*

The PBDEs in water samples were extracted with solid-phase extraction (SPE) columns (LC-C18) (Milwaukee, WI, USA) following the procedures described by Wang et al. (2011). Briefly, water samples were passed through glass-fiber filters (0.45 μm; Whatman, Clifton, NJ, USA) to get rid of the particulates and sonicated in 40% methanol (v/v) for 30 min. The water samples were then purified by the preconditioned SPE columns eluting with 3 mL of methanol, 3 mL of dichloromethane and 3 mL of *n*-hexane. The collected extracts were further cleaned on a 30 cm × 10 mm (i.d.) glass column, which was packed from bottom to top with 6 cm neutral alumina, 2 cm neutral silica, 5 cm alkalinized silica, 2 cm neutral silica, 8 cm acidified silica, and 1 cm Na_2SO_4. The PBDEs mixture was eluted with 70 mL of 50% DCM in hexane and the final extract was concentrated with a rotary evaporator, dried under gentle nitrogen gas and re-dissolved in 0.6 mL isooctane. A known amount (4 ng) of internal standard (^{13}C12-labeled PCB208) was added to all extracts prior to subsequent analysis by gas chromatography/mass spectrometry (GC/MS).

Sample analysis were carried out with an Agilent GC 6890N coupled with 5975C MS (Agilent Technologies, Wilmington, DW, USA), using electron ionization in the selected ion monitoring mode with helium as the carrier gas at the flow rate of 0.8 mL/min. A DB-5HT MS fused silica capillary column (15 m × 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific) was used for the determination of PBDE congeners with auto splitless injection of 1 μL sample. The ion source and interface temperatures were set to 230 °C and 280 °C, respectively. Procedure blanks was included for each set analysis of 8 samples. Recoveries of PBDE congeners ranged from 75 to 110% in the

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