Comparative Biochemistry and Physiology, Part C 152 (2010) 62–68

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Teratogenic effects of tetrabromobisphenol A on *Xenopus tropicalis* embryos

Huahong Shi *, Lijuan Qian, Suzhen Guo, Xiaoli Zhang, Junqi Liu, Qinzhen Cao

Department of Environmental Science, Key Laboratory of Urbanization and Ecological Restoration, East China Normal University, Shanghai 200062, China

ARTICLE INFO

Article history:
Received 10 January 2010
Received in revised form 21 February 2010
Accepted 21 February 2010
Available online 24 February 2010

Keywords:
Amphibian
Brominated flame retardants
Thyroid hormone
Teratogenicity

ABSTRACT

Tetrabromobisphenol A (TBBPA) is the most widely used brominated flame retardant and a known thyroid disruptor. We reported exposing *Xenopus tropicalis* embryos (NF10) to 0.01, 0.1 or 1 mg/L of TBBPA with or without 70 μg/L triiodothyronine (T_3). Compared with the controls, 1 mg/L of TBBPA significantly reduced the body length of embryos after 24, 36 and 48 h of exposure. Embryos treated with TBBPA showed multiple malformations, including: abnormal eyes, skin hypopigmentation, enlarged proctodaeum, narrow fins and pericardial edemas. The effect of abnormal eyes manifested itself in the loss of pigmentation, reduction in size, or absence of external eyes. The degree of eye malformations was quantified with the index of eye malformations (IEM) with 0 being normal and 3 being severe. In the 1 mg/L TBBPA treatment groups, the incidence of total malformations (ITM) was 68–93%, and IEM was 0.8–0.9. T_3 showed no teratogenic effects on embryos, but it significantly enhanced TBBPA-induced teratogenic effects. In the $T_3 + 1$ mg/L TBBPA treatment groups, ITM was 91–99%, and IEM was 1.8–1.9. Histological observations showed that the retinas were generally smaller, and the lenses were underdeveloped or even absent. These results indicate that TBBPA at relatively high concentration has teratogenic effects on *X. tropicalis* embryos. The results also suggest that thyroid hormone signaling might be involved in TBBPA induced-teratogenicity.

1. Introduction

Brominated flame retardants (BFRs) are important chemicals used in electronic equipment, plastics, and building materials (Alaee et al., 2003). Tetrabromobisphenol A (TBBPA) is the most widely used BFR on the market and has been observed in several kinds of environments all over the world (Morris et al., 2004; Covaci et al., 2009). The highest concentration of TBBPA in the environment documented in literature is 0.62 μg/L (Osako et al., 2004). The European Union (EU) concludes that TBBPA shows no risk to human health or the environment (European Commission, 2008). However, this does not assess the effects of higher levels of exposure found outside the EU such as around sites of manufacturing and where reactive TBBPA is used (Law, 2009). Therefore, there is still heightened concern regarding the toxic effects of TBBPA and the mechanisms of action (Shi et al., 2005; Covaci et al., 2009).

TBBPA has been shown to disrupt thyroid hormones (THs) in *in vivo* and *in vitro* assays (Kitamura et al., 2002, 2005; Veldhoen et al., 2006; Fini et al., 2007). Due to the structural resemblance to thyroid hormones, TBBPA can bind to transthyretin (TTR) and thyroid hormone receptors (TRs) (Kitamura et al., 2002). In recent years, concern about thyroid disrupting chemicals (TDCs) has increased because THs play a critical role during the development of animals (Miller et al., 2009). Currently, amphibian metamorphosis has been widely used as a model to screen TDCs (Degitz et al., 2005; Fort et al., 2006; Opitz et al., 2006). Some studies suggest that TBBPA acts as a TH antagonist in frogs. For example, TBBPA inhibits larval development of *Xenopus laevis* during long-term exposure (Jagnytsch et al., 2006), and shows suppressive action on T_3-enhancement of *Rana rugosa* tadpole tail resorption (Kitamura et al., 2005). Other studies, however, suggest that TBBPA can act as a TH agonist in frogs. For example, TBBPA enhances TH-mediated effects and increases the expression of TRα mRNA in Pacific tree frog *Pseudacris regilla* (Veldhoen et al., 2006).

Triiodothyronine (T_3) is the active form of THs and acts through TRs, which are transcription factors regulating gene expression. A common mechanism for a wide variety of TDCs is interference with TH signaling by changing intracellular TH availability or by interacting directly with the level of TRs. Recent research suggests that TH signaling is also active in early amphibian embryos (Cossette and Drysdale, 2004; Morvan-Dubois et al., 2006). Morvan-Dubois et al. (2008) proposed that both unliganded and liganded TRs can have active roles, and that ligand availability is a determining factor orchestrating the actions in *X. laevis* embryos. This means that some chemicals might disturb TH signaling not only during the metamorphic stage but also during the early embryogenic stage. In *Xenopus* embryos, iopanoic acid (a deiodinase inhibitor) and methimazole (a thyroid peroxidase inhibitor) have been proved to induce teratogenicity by changing TH availability; NH-3 (a TRs antagonist) and overexpression of TR mRNA have been proved to induce eye malformations by interacting with TRs (Havis et al., 2006; Tindall et al., 2007). In

* Corresponding author. Tel.: +86 21 62238338; fax: +86 21 62232374.
E-mail address: hhshi@des.ecnu.edu.cn (H. Shi).

1532-0456/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2010.02.013

addition, 10 or 100 nM of T_3 shows no teratogenic effects on *X. laevis* embryos, but T_3 significantly enhances iopanoic acid (IOP) or TR mRNA-mediated eye malformations (Puzianowska-Kuznicka et al., 1997; Havis et al., 2006). With the exception of these pharmacological agents, there are no other industrial chemicals reported to disrupt TH signaling during the amphibian embryogenic stage.

X. tropicalis is an emerging animal model in developmental biology. It is highly related to *X. laevis* and has more advantages such as smaller size and shorter life cycle. In this paper, we exposed embryos of *X. tropicalis* to TBBPA in the absence or presence of T_3. Our aim was to determine if TBBPA shows adverse effects on the development of *X. tropcialis* embryos, and to determine if TH signaling is involved in these effects.

2. Materials and method

2.1. Chemicals

TBBPA (4,4′-isopropylidenebis(2,6-dibromophenol); 97%), T_3 (3,5,3′-triiodothyronine), 3-amino-benzoic acid ethyl ester (MS-222), and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in this study were analytical grade.

2.2. Husbandry of X. tropicalis

X. (*Silurana*) *tropicalis* were obtained from Nasco (Fort Atkinson, WI, USA). Mature female and male frogs were maintained separately in plastic tanks with a 12 h light/12 h dark cycle. The water was dechlorinated with activated charcoal filters. The water conditions were: temperature, 26 ± 0.5 °C; dissolved oxygen, >6 mg/L; total hardness, 150–250 mg/L of $CaCO_3$; pH, 7.2–7.8. The frogs were fed three times a week on frog brittle (Purina Jiaxing Co., Ltd, Zhejiang, China). Breeding was induced by subcutaneous injection of human chorionic gonadotrophin (hCG) (Ningbo Renjian Pharm. Co., Ltd, Zhejiang, China) in the dorsal lymph sac. Both males and females were injected as follows: 20 IU hCG for each at 9–10 am of the day one, and 100 IU hCG for each at 9–10 pm of day two.

2.3. Exposure experiments

The exposure experiments were based on the frog embryo teratogenesis assay-*Xenopus* (FETAX) with some modifications (ASTM, 1998). In brief, adults were removed on the second morning after the injections, and the embryos were harvested without removing their jelly coats. TBBPA and T_3 were dissolved in DMSO (<0.1%). Embryos at NF Stage 10 were exposed to 0.01, 0.1 or 1 mg/L of TBBPA in the absence or presence of 70 μg/L of T_3 (Nieuwkop and Faber, 1956). In addition, a solvent control was run. Twelve dishes were performed for each control or treatment group.

Twenty healthy embryos were carefully chosen under dissecting microscope and put in an acid-washed glass Petri dish (10 cm diameter) with different media. The dishes were incubated at 26 ± 0.5 °C with 24 h of darkness. The dead embryos were removed, and the media were renewed at 24 h intervals. At 24, 36 and 48 h after exposure, 4 dishes of embryos were sampled from each group. The surviving embryos were anaesthetized with 100 mg/L of MS-222 and fixed with 4% formalin for 24 h. The embryos were then washed with tap water and preserved with 70% ethanol.

2.4. Observation and measurements of embryos

Embryos were observed with an Olympus SZX16 dissecting microscope (Olympus Corporation, Japan), and images were taken with an Olympus DP 25 camera (Olympus Corporation, Japan). The body length was measured from the tip of the head to the tip of the tail using computer-assisted image analysis. The types of malformations were identified, and the number of malformations was recorded. Eye malformations were classified based on the pigmentation, shape and size of the eyes. The degree of eye malformations was graded on a 0 to 3 scale with 0 being normal and 3 being the absence of external eyes.

2.5. Histology

To determine the interior changes to the malformed eyes, 15–20 embryos were randomly selected from each grade (0–3) for histological sectioning. The fixative was removed by rinsing in running tap water and soaking in 70% ethanol. The embryos were wax embedded and transversely sectioned (6 μm) through the heads. The slides were then stained with hematoxylin-eosin and observed with a Nikon 801 microscope.

2.6. Statistical analysis

Statistical analysis was conducted with the aid of SPSS 13.0 software. Each dish of 20 embryos was considered a replicate, and there were 4 replicates per each group ($n = 4$). Differences were analyzed between DMSO control and treatment groups, and between TBBPA and TBBPA + T_3 treatment groups. Dish-to-dish variation was handled using one-way ANOVA, followed by Dunnett's test.

3. Results

3.1. Effects of TBBPA and T_3 on survival and growth of X. tropicalis embryos

Compared with DMSO controls, T_3 showed no effect on the survival rate of embryos at any sampling time. In the 1 mg/L TBBPA treatment groups, the survival rate was decreased by 6% after 24 h of exposure in the absence of T_3, and by 13% after 36 h and by 10% after 48 h in the presence of T_3 (Fig. 1A–C). The body length of the embryos was decreased in the groups treated with T_3 by 1.8–2.4% after 36 and 48 h of exposure. Also, the body length was decreased by 7.4–19.3% in the groups treated with 1 mg/L of TBBPA in the absence or presence of T_3 (Fig. 1D–F). Compared with the TBBPA treatment groups, the body length was decreased in the T_3 + 0.1 mg/L TBBPA after 36 h and the T_3 + 0.01 or 0.1 mg/L TBBPA treatment ones after 48 h of exposure.

3.2. Teratogenic effects of TBBPA and T_3 on X. tropicalis embryos

Most of the embryos showed normal morphology in the DMSO control and DMSO + T_3 treatment groups. Multiple phenotypes of malformations were found in the TBBPA treatment groups with or without T_3. After 24 h of exposure, the most characteristic malformations were abnormal eyes and bent tails (Fig. 2). The effect of abnormal eyes manifested itself in the loss of pigmentation, variation in size, or absence of external eyes (Fig. 2D). After 36 h of exposure, the most frequent malformations were abnormal eyes, skin hypopigmentation, pericardial edemas, enlarged proctodaeum, and narrow fins (Fig. 3). After 48 h of exposure, the primary phenotypes of malformations became much more severely pronounced in the treatment groups. In addition, enlarged trunks and bent notochord were observed (Fig. 4).

The incidence of total malformations (ITM) was no more than 5%, and the index of eye malformations (IEM) was no more than 0.04 in the DMSO control or DMSO + T_3 treatment groups (Fig. 5). There was no significant difference in ITM or IEM between DMSO + T_3 treatment and DMSO control groups. Compared with the DMSO controls, 1 mg/L of TBBPA greatly increased ITM (68–93%) and IEM (0.8–0.9) (Fig. 5). In TBBPA treatment groups, ITM and IEM were further increased by the presence of T_3. ITM ranged from 91% to 99%, and IEM ranged from 1.8 to 1.9 in the groups treated with