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Intranasal administration of tetrabromobisphenol A bis(2-hydroxyethyl ether) induces neurobehavioral changes in neonatal Sprague Dawley rats

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ABSTRACT

Tetrabromobisphenol A (TBBPA) and its derivatives are now being highly concerned due to their emerging environmental occurrence and deleterious effects on non-target organisms. Considering the potential neurotoxicity of TBBPA derivatives which has been demonstrated *in vitro*, what could happen *in vivo* is worthy of being studied. Tetrabromobisphenol A bis(2-hydroxyethyl ether) (TBBPA-BHEE), a representative TBBPA derivative, was selected for a 21-day exposure experiment on neonatal Sprague Dawley (SD) rats through intranasal administration. The neurobehavioral, histopathological changes, and differentially expressed genes based on RNA microarray were investigated to evaluate the neurological effects of this chemical. The results indicated that TBBPA-BHEE exposure significantly compromised the motor co-ordination performance and the locomotor activities ($p < 0.05$). The neurobehavioral phenotype could be attributed to the obvious histopathological changes in both cerebrum and cerebellum, such as neural cell swelling, microglial activation and proliferation. A total of 911 genes were up-regulated, whereas 433 genes were down-regulated. Gene set enrichment analysis showed multiple signaling pathways, including ubiquitin-mediated proteolysis and wingless-int (Wnt) signaling pathway *etc.* were involved due to TBBPA-BHEE exposure. The gene ontology enrichment analysis showed the basic cellular function and the neurological processes like synaptic transmission were influenced. The toxicological effects of TBBPA-BHEE observed in this study suggested the potential neuronal threaten from unintended exposure, which would be of great value in the biosafety evaluation of TBBPA derivatives.

Introduction

Tetrabromobisphenol A (TBBPA) are the most widely utilized brominated frame retardants (BFRs) worldwide, and accounts for about 60% of the total BFR market (A.F. Liu et al., 2016). Wherein, the manufacture of TBBPA derivatives has occupied approximate 18% of TBBPA production (Liu et al., 2015). The prevalent forms, including tetrabromobisphenol A bis(2-hydroxyethyl ether) (TBBPA-BHEE), tetrabromobisphenol A bis(glycidyl ether) (TBBPA-BGE), tetrabromobisphenol A bis(allylether) (TBBPA-BAE) and tetrabromobisphenol A bis(dibromopropyl ether) (TBBPA-BDBPE), are commonly used

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in engineering polymers or as precursors for other TBBPA-based polymers (Qu et al., 2013), and their annual demands have been continually increased (Tian et al., 2015). Nevertheless, the concomitant unintended release of the related compounds during the production, application, and disposal, brings the new environmental issues (Qu et al., 2013; A.F. Liu et al., 2016), potentially causing deleterious influences on multiple non-target organisms like bacterial community (Zhang et al., 2015), aquatic species (Liu et al., 2007), rodents (Szymanska et al., 2000), and even human beings (Colnot et al., 2014).

TBBPA has been found to induce diverse toxicological effects according to *in vitro* and *in vivo* studies (Nakajima et al., 2009; Reistad et al., 2007). As well as thyroidogenic, estrogenic, and dioxin-like activity (Birnbaum and Staskal, 2004; Wang et al., 2017), neurotoxicity has been studied for TBBPA, and the results indicated that this compound induce apoptosis and toxicity in mouse primary neuronal cells cultures (Wojtowicz et al., 2013), inhibited plasma membrane uptake of the neurotransmitters (Mariussen and Fonnum, 2003), impaired habituation of motor activity and spatial learning in adult rats (Hass and Wamberg, 2002). A single neonatal exposure to TBBPA affected neurodevelopment and synaptic plasticity to a small extent in mice (Hendriks et al., 2015). Increasing concerns are being drawn on the potential deleterious impacts of TBBPA derivatives, considering their structural similarity to TBBPA. Nevertheless, the knowledge on the toxicological data of TBBPA derivatives still remains sparse, and is highly required to cope with the emerging exposure risks.

Considering the extensive environmental exposure risk (Esch, 1995) due to the expanding annual sale volumes, TBBPA-BHEE has been registered by the Danish Environmental Protection Agency inventory list (Andersson et al., 2006). Previous *in-vitro* study reported that TBBPA-BHEE exhibited relatively higher cellular toxicity than the parent compound, TBBPA and the other three derivatives TBBPA derivatives, and its stimulation caused neurotransmitter dysfunction and ROS-mediated caspase activation in rat pheochromocytoma cell (PC12), resulting in the eventual neurotoxicity (Q. Liu et al., 2016). The question whether TBBPA-BHEE could induce *in-vivo* neurotoxicological effects, would be of great importance in explaining its potential neural hazards.

Since neonatal Sprague Dawley (SD) rats were sensitive to exogenous stress and drug delivery through intranasal instillation was brain-targeted (Wen et al., 2016; Win-Shwe et al., 2014), we investigated the neurological effects of TBBPA-BHEE on rotarod performance and open-field activity after 21-day treatment in neonatal SD rats through intranasal instillation in this study. The histopathological changes and differentially expressed gene profile were evaluated to elucidate the hazardous effects of TBBPA-BHEE on the brain at cellular and molecular levels.

1. Materials and methods

1.1. Chemicals and reagents

TBBPA-BHEE (CAS 4162–45-2, 98%) was obtained from Sigma-Aldrich (USA). The stock solution was made by dissolving TBBPA-BHEE in dimethyl sulfoxide (DMSO). The series of working solutions were diluted with sterile water and the final DMSO concentration was less than 1%. All other chemicals or reagents were purchased from Sigma-Aldrich (USA), unless stated otherwise.

1.2. Animals

The pregnant SD rats (around 18 days after conception, $n = 4$) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (China), and kept in Peking University Health Science Center for the neonate delivery and the following rearing. After approximate 1-week acclimatization, total 63neonatal rats (34 male, and 29 female) were obtained and raised with their mother for the normal breast milking until day 21. Only male neonatal rats were used for the experiment to avoid the possible gender interference. Sterile water and SPF feed (Vital River Laboratories, China) were provided *ad libitum*. The animal room was maintained at 25 °C,*ca.*60% of relative humidity, and 12 hr/12 hr light/dark cycle. All animals were kept according to the principles of care and use of laboratory animals approved by the Institutional Animal Care and Use Committee of Peking University.

1.3. Exposure protocols

The exposure was initiated for the male neonatal rats on the 2nd day after birth, and the animals from 4 broods with the average body weight of around 6 g, were randomly divided into 4 groups ($n = 8$), including distilled water, 1% DMSO (vehicle control group), 0.086 mg/kg of TBBPA-BHEE(low-dose exposure group) and 0.86 mg/kg of TBBPA-BHEE (high-dose exposure group).The exposure doses were200 and 2000 folds lower than theLD_{50} (180 mg/kg) of TBBPA-BHEE in rats through intraperitoneal injection (Esch, 1995) to study the potential nonlethal effect. The exposure through intranasal instillation was performed once a day at the dose of 1 μL/g, and lasted continuously for 21 days. The physiological changes including body weight, water drinking, food uptake, animal behavior and death were daily observed during the experiment. On the 3rd week of exposure, the rats from each group were submitted to the neurobehavioral tests, including rotarod and open field experiments. After the tests, all the animals were sacrificed by CO_2inhalation, and the cerebellum and cerebrum samples were harvested after intracardiac perfusion by 30 mL of ice-cold PBS. For the histopathological studies, the samples were collected from the animals ($n = 3$ from each group) with the subsequent perfusion of 30 mL of PBS containing 10% formaldehyde. In regard of RNA microarray analysis, the samples were obtained from the animals ($n = 3$ from vehicle control and high-dose exposure groups) with the following perfusion of 30 mL of ice-cold 10% RNA later (Thermo, USA).Due to the limitations in sensitivity and matrix effects of currently available analytical methods for TBBPA-BHEE (Wang et al., 2013; Tian et al., 2015), the exposure concentrations were used for the evaluation of this chemical's neurotoxicity in this study.

1.4. Rotarod test

The rotarod test was performed during the 3rd week. The rats from each group were pre-trained for 3 trials in sequence to

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