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In vitro assessment of thyroid hormone receptor activity of four organophosphate esters

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ABSTRACT

Previous animal experiments have implied that organophosphate esters (OPEs) have a disruption effect on the thyroid endocrine system. However, knowledge of the toxicological mechanism remains limited. In this study, the activities of four OPEs have been characterized against the thyroid hormone (TH) nuclear receptor (TR) using two *in vitro* models, with the aim of evaluating their toxicity mechanisms towards the TR. The results of a TH-dependent cell proliferation assay showed that tris(2-chloro-1-(chloromethyl)ethyl)phosphate (TDCPP) could induce cell growth, while the other three OPEs had no effect. The results of a luciferase reporter gene assay revealed that all four of the OPEs tested in the current study showed agonistic activity towards TRβ, with TDCPP being the most potent one. Moreover, molecular docking revealed that all the tested OPEs could fit into the ligand binding pocket of TRβ, with TDCPP binding more effectively than the other three OPEs. Taken together, these data suggest that OPEs might disrupt the thyroid endocrine system *via* a mechanism involving the activation of TR.

Introduction

Organophosphate esters (OPEs) are the esters of phosphoric acids, and these compounds can be mainly classified into trialkyl-, trichloroalkyl- and triaryl-phosphates according to the nature of their substituent groups (Reemtsma et al., 2008). OPEs have been used as flame retardant additives and plasticizers in various consumer products, including building materials, electronic devices, plastic products, textiles and baby products (van der Veen and de Boer, 2012). Given that OPEs are not covalently bound to the host materials, these compounds tend to migrate into the surrounding environment, and can ultimately make their way to the human body (Bollmann et al., 2012). In recent years, the production and use of OPEs, which were proposed as alternatives of brominated flame retardants (BFRs), have increased significantly because of plans to ban and phase-out the use of BFRs (Wei et al., 2015). OPEs have consequently become ubiquitous environmental contaminants, and increasing concentrations of compounds belonging to this structural class have been detected in water, indoor dust and outdoor air (Meeker and Stapleton, 2010; Salamova et al., 2014). Furthermore, OPEs have been detected in the human body (Wei et al., 2015). Because of the widespread exposure, there has been growing concern regarding the potential harmful effects of OPEs on human health.

Toxicology studies have shown that exposure to OPEs has the potential to cause developmental toxicity (McGee et al., 2012), neurotoxicity (Wang et al., 2015a, 2015b), adverse reproductive

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issues (Liu et al., 2013b), disruption to the endocrine system (Liu et al., 2012) and a range of other systemic effects in experimental animal models (Dishaw et al., 2014). However, knowledge of the toxicological mechanisms of OPEs remains limited. To date, there have been numerous mechanistic studies focused on the toxicities of OPEs as a consequence of their interactions with nuclear receptors pathways, which have been shown to mediate the disruptive effects of many other pollutants (Ren et al., 2013). Based on the results of three *in vitro* models, Zhang et al. (2014) demonstrated that OPEs interfered with the estrogen receptor (ER) pathway, with three OPEs had remarkable anti-estrogenic properties. However, contrary results were obtained in another study, where OPEs were found to be ER antagonists (Liu et al., 2012). Furthermore, the human aryl hydrocarbon receptor (AhR), human constitutively active receptor and human pregnane X receptor (PXR) pathways were found to be affected by OPEs (Gerlach et al., 2014; Honkakoski et al., 2004). The results of two recent studies suggested that some of these OPE compounds could exert their toxicity via the activation of the peroxisome proliferator-activated receptor (PPARγ) pathway (Belcher et al., 2014; Fang et al., 2015). Furthermore, Kojima et al. (2013) evaluated the effects of OPEs against eight human nuclear receptor pathways and found that four of these pathways were affected by the OPEs:ER agonistic activity, androgen receptor antagonistic activity, glucocorticoid receptor antagonistic activity and PXR agonistic activity. No effects were observed for the other four nuclear receptor pathways (thyroid hormone receptor (TR), retinoic acid receptor (RAR), retinoid X receptor (RXR) and PPARγ) (Kojima et al., 2013). Given that the data obtained to date remain scattered and sometimes contradictory, further work is required to understand the disruptive impact of OPEs on the nuclear receptor pathways.

Thyroid hormones (THs) play critical roles in many biological functions by regulating the expression of target genes controlled by TRs including the subunit of TRα and TRβ. The disruption of the thyroid endocrine system by OPEs has been reported in previous studies. For example, the results of a human epidemiological survey conducted in the USA suggested that elevated levels of OPEs in house dust could be associated with altered TH levels (Meeker and Stapleton, 2010). Moreover, the results of numerous animal experiments have indicated the disruptive effect of OPEs on the thyroid endocrine system. Wang et al. (2013, 2015a, 2015b) found that TDCPP changed TH levels and altered the transcription of genes involved in the hypothalamic–pituitary-thyroid (HPT) axis in zebrafish embryos/larvae. Liu et al. (2013a) evaluated the effects of TDCPP and TPP on six receptor-associated expression of mRNA in zebrafish embryos/larvae, and the results showed that TH receptor-centered gene networks were altered by OPEs. Using real-time reverse transcription-PCR, it has been demonstrated that exposure to TDCPP and TCPP altered the mRNA abundance of genes associated with the TR pathway in cultured hepatocytes and neuronal cells derived from embryonic chickens (Crump et al., 2012; Farhat et al., 2013). According to the results of previous studies, TR pathway appears to be interfered by OPEs. To the best of our knowledge, there has been only one paper reported in the literature pertaining to the activities of OPEs towards the human TR, where none of the test compounds showed any agonistic or antagonistic activity (Kojima et al., 2013). Given that different results were obtained from different studies, such as the conflicting results described above for the activities of OPEs towards the ER and PPARγ, it is still necessary to study the effects of OPEs towards the TR to determine the toxicity mechanisms of these compounds.

In this study, we assessed the activity of four OPEs (Fig. 1a) towards the TR using a TH-dependent cell proliferation assay and a luciferase reporter gene assay. Molecule docking experiments were also employed to simulate the interactions between these compounds and the TRβ ligand binding domain (LBD) in an attempt to understand the structural basis for the experimentally observed activities of these compounds.

1. Materials and methods

1.1. Chemicals

Four OPEs including trimethyl phosphate (TMP), triethyl phosphate (TEP), tris(2-chloroethyl)phosphate (TCEP) and TDCPP were purchased from Dr. Ehrenstorfer Gmbh (Augsburg, Germany). The OPEs were dissolved in dimethyl sulfoxide (DMSO) to give 50 mmol/L stock solutions. 3,5,3′-Triiodothyronine (T3) was purchased from Fitzgerald Industries International, Inc. (Concord, MA, USA). Amiodarone was purchased from Sigma-Aldrich (St. Louis, MO, USA). All of the other reagents were purchased as the highest available purity.

1.2. GH3 cell proliferation assay

We screened the effects of the OPEs towards the TR pathway by examining the proliferation of a TH-dependent rat pituitary tumor cell line (GH3). The experimental details for T-screen assay are in the Supplementary data. GH3 cells were exposed to the chemicals at concentrations in the range of 1–100 μmol/L to evaluate the activities of the OPEs on TR pathway.

1.3. Transient transfection-based luciferase reporter gene assay

A cell-based human TRβ-driven luciferase reporter assay was developed to determine the activities of the OPEs towards the human TRβ. The experimental details for luciferase reporter assay are in the Supplementary data. In the agonistic potency assay, the cells were exposed to different concentrations (1–100 μmol/L) of OPEs as well as T3 (0.2–50 nmol/L) alone to test their agonistic activities. The antagonistic potency was determined by treating cells with OPEs in the presence of 2 nmol/L T3.

1.4. Molecular docking

AutoDock 4.2 (La Jolla, CA, USA) was used to simulate the interactions between the OPEs and TRβ-LBD. The details of the docking procedures are described in the Supplementary data.

1.5. Statistical analysis

The *p* values of the experimental data were analyzed by two-way ANOVA followed by Duncan post hoc analyses ($p < 0.05$). A *p* value of less than 0.05 was considered statistically significant. All of the experiments were conducted in triplicate, and the data