Toxicology in Vitro 29 (2015) 44–50

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Modulation of estradiol synthesis and aromatase activity in human choriocarcinoma JEG-3 cells exposed to tetrabromobisphenol A

Ewelina Honkisz, Anna K. Wójtowicz *

Department of Animal Biotechnology, University of Agriculture, Rędzina 1B, 30-248 Cracow, Poland

ARTICLE INFO

Article history:
Received 18 March 2014
Accepted 5 September 2014
Available online 16 September 2014

Keywords:
JEG-3
Tetrabromobisphenol A
Estradiol
Aromatase
cAMP
Cytotoxicity

ABSTRACT

The goal of the present study was to investigate the impact of tetrabromobisphenol A (TBBPA) on human choriocarcinoma-derived placental JEG-3 cells *in vitro*. We determined the effect of this compound on estradiol secretion, aromatase protein expression and activity *in vitro* in the JEG-3 cell line. We assessed the ability of TBBPA to increase intracellular levels of cAMP as well as its effect on cell viability and proliferation. Our results indicated that TBBPA, at a wide range of concentrations (1×10^{-8}–5×10^{-5} M), significantly induced estradiol secretion by JEG-3 cells compared to that of controls after 24, 48 or 72 h of exposure. This effect was accompanied by an increase in the aromatase protein expression in JEG-3 cells treated with 100 nM and 10 μM of TBBPA for 24 h. Additionally, in our study, we confirmed that TBBPA-induced changes in aromatase protein expression were associated with the up-regulation of aromatase activity and cAMP levels. No tested doses of TBBPA inhibited JEG-3 cell proliferation, except for the highest dose of 100 μM, which had a toxic effect on cell viability at all time points. The present study clearly indicates that TBBPA alters JEG-3 cells estrogen synthesis due to its action on CYP19 protein expression and thus this compound may interfere with normal placental development during early pregnancy.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Tetrabromobisphenol A (TBBPA) is the most widely used compound among brominated flame retardants (BFRs) in industrial and consumer products (de Wit, 2002). World production of TBBPA is mainly focused in the United States, Israel and Japan and is estimated to be close to 200,000 tons per year (Congleton, 2013). When considering production data for 1992 and 1999, which were 50,000 and 121,300 tons per year, respectively, the market demand for TBBPA is steadily increasing (de Wit, 2002). This chemical is applied as a flame retardant in a wide variety of commercial and household products, from which TBBPA is released and circulates as dust throughout homes, offices and the interior of passenger cars (Abdallah et al., 2008; Geens et al., 2009). The highly lipophilic character of this substance, and thus its ease of crossing cell membranes, should be a matter of greater concern. That widespread use is leading to chronic human exposure to TBBPA is supported by numerous epidemiological studies, which have noted its presence in human body fluids, such as blood (Dirtu et al., 2008; Hagmar and Bergman, 2001; Jakobsson et al., 2002; Nagayama et al., 2001; Thomsen et al., 2007) and breast milk (Cariou et al., 2008; Shi et al., 2009; Thomsen et al., 2002). Moreover, TBBPA is easily transported through the placental barrier, as it has been detected in umbilical cord tissue (Antignac et al., 2008; Kawashiro et al., 2008; Zalko et al., 2007). The potential endocrine-disruptive action of TBBPA both *in vitro* and *in vivo* is well documented. Its endocrine-related biological activity has mostly been supported through interactions with the thyroid hormone system in both rats and humans, primarily through strong competitive binding to transthyretin and thyroid hormone receptors (Kitamura et al., 2002; Meerts et al., 2000; Van der Ven et al., 2008). Additionally, there are some data available regarding disorders of reproductive processes caused by the impact of TBBPA on the proliferation of rat pituitary gland cells and the improper synthesis of growth hormone (Kitamura et al., 2002, 2005). The compound also affected adrenal steroidogenesis in H295R cells (Canton et al., 2005; Song et al., 2008). Despite the widespread occurrence of TBBPA in the environment, limited information is available on its estrogenic effects. TBBPA had a positive effect in an *in vivo* uterotrophic assay using ovariectomized mice (Kitamura et al., 2005) and also inhibited estrogen sulfotransferase *in vitro* (Hamers et al., 2006; Kester et al., 2002).

To date, there are no data indicating whether TBBPA affects human placental function in early pregnancy. Thus, the aim of the present study was to investigate the effect of TBBPA on placental secretion of estradiol and to determine the effect of this compound

* Corresponding author. Tel./fax: +48 12 429 75 47.
E-mail address: anna.wojtowicz@ur.krakow.pl (A.K. Wójtowicz).

http://dx.doi.org/10.1016/j.tiv.2014.09.003
0887-2333/© 2014 Elsevier Ltd. All rights reserved.

on aromatase protein expression and activity in the JEG-3 cell line *in vitro*. Additionally, we analyzed the effect of TBBPA on cell viability and proliferation. The human choriocarcinoma-derived placental JEG-3 cell line is a reliable model in studies of placental function; it possesses many biological and biochemical characteristics of syncytiotrophoblasts (Matsuo and Strauss, 1994), produces placental hormones (Chou, 1982; Kato and Braunstein, 1991) and expresses enzymes involved in steroidogenesis (Sun et al., 1998; Tremblay et al., 1999); therefore, it is used to examine the hormonal function of trophoblasts and intracellular receptor mechanisms. In addition, JEG-3 cells exhibit high aromatase activity (Moise et al., 1986; Ritvos, 1988). Overall, this cell line is a well-known *in vitro* model to assess and understand endocrine disruption by environmental chemicals.

2. Materials and methods

2.1. Cell culture and toxicant treatment

The human choriocarcinoma-derived placental JEG-3 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in DMEM without phenol red, supplemented with 10% charcoal-stripped FBS (and thus depleted of steroid hormones), 100 UI/ml penicillin and 100 g/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

Cells were seeded in 96-well culture plates (Costar, St. Louis, MO, USA) at a density of 8×10^3 (for the 24 h treatment), 7×10^3 (for the 48 h treatment) or 6×10^3 (for the 72 h treatment) and initially cultured for 24 h. Subsequently, the medium was changed to DMEM supplemented with 5% charcoal-stripped FBS in the presence of TBBPA (1 nM, 10 nM, 50 nM, 100 nM, 1 μM, 10 μM, 50 μM, or 100 μM). These concentrations are in the range of TBBPA concentrations reported in maternal and cord serum (Cariou et al., 2008; Shi et al., 2009). For example, the concentration of TBBPA in maternal serum ranged from 0.06 to 37.34 ng/g lipid weight, which is equal to 109 pM–69 nM. Cord serum ranged from 2 to 649 ng/g lipid weight, which is equal to 3 nM–1 μM. Although TBBPA concentrations at the micromolar range (10, 50 and 100 μM) are higher than those found in the body fluids, this chemical may exert its effect through chronic exposure or condensation through the food chain (Fujii et al., 2014; Shi et al., 2009).

Each stock solution of TBBPA was made in DMSO as a 1000-fold stock solution and stored at 4 °C. For cell exposure, the incubation media were prepared through a 1000-fold dilution of DMSO stock solution with the culture medium (0.1% vol/vol DMSO) just prior to cell dosing. At this concentration, DMSO has no effect on steroid secretion or cell viability, the latter of which was determined before seeding by the Trypan blue exclusion test and found to be ⩾95% (data not shown). The control group was cultured in a 0.1% DMSO medium.

2.2. Cytotoxicity assay

Cytotoxicity was assessed with a cytotoxicity detection kit (Roche Applied Science, Mannheim, Germany) according to manufacturer's instructions. Briefly, after 24, 48 or 72 h of exposure to increasing doses of TBBPA, 100 μl of culture medium was transferred to 96-well microtiter plates, and lactate dehydrogenase (LDH) activity was determined by the addition of the substrate solution. Formazan formation was measured at 492 nm in a microplate reader (Bio-Tek ELx800). The data were analyzed with KCJunior (Bio-Tek Instruments) and normalized to the absorbance of vehicle-treated cells. The results are expressed as the mean absorbance of ten separate samples ± SEM. Experiments were performed at least in triplicate. Extinction values of control cells were set at 100%, and the rate of LDH release from treated cells was calculated as a percentage of the control.

2.3. AlamarBlue® assay for JEG-3 proliferation

To assess whether TBBPA affected JEG-3 cell proliferation, the fluorescent dye alamarBlue® (AbD Serotec) was used. This water-soluble dye has been previously applied in various *in vitro* cell studies (Ansar Ahmed et al., 1994; Fields and Lancaster, 1993). Moreover, the dye is not toxic to cells, and continuous monitoring of the cell culture is possible. This method is based on the detection of metabolic activity. The active ingredient of alamarBlue® is resazurin. It is blue in color and virtually a non-fluorescent agent. After entering cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent. The intensity of the red color reflects the extent of cellular proliferation. The proliferation assay was performed as described previously (Honkisz et al., 2012). The data were analyzed with KCJunior (Bio-Tek Instruments) and normalized to the fluorescence of vehicle-treated cells. The results are expressed as the percentage of control fluorescence from ten separate samples ± SEM, which were run at least in triplicate. The fluorescence values of blanks, as determined using culture medium with 10% alamarBlue® without cells, were subtracted.

2.4. Estradiol analysis

Human choriocarcinoma cells, similar to normal placenta, appear to lack significant 17β-hydroxylase and 17-20-desmolase activity (Bahn et al., 1981). The absence of an enzyme that catalyzes the conversion of pregnenolone to DHEA makes these cells unable to synthesize estradiol *de novo* from cholesterol, similar to ovarian cells. Thus, to study the dose- and time–response effects of different TBBPA doses on estradiol secretion, the cells were cultured in DMEM supplemented with 500 ng/ml dehydroepiandrosterone (DHEA) (Zbella et al., 1986). After an appropriate exposure time (24, 48 or 72 h) to the complete range of concentrations of TBBPA, the media was frozen at −20 °C for estradiol analysis. Each treatment was analyzed in ten wells, and each experiment was repeated three times. The concentrations of estradiol were determined by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (DRG, Marburg, Germany) according to the manufacturer's instructions. The sensitivity of the estradiol assay was 9.714 pg/ml, and the intra- and inter-assay CV values were 2.71% and 6.72%, respectively. The sample concentrations were calculated using a best-fit four-parameter logistic calibration curve (KCJunior; BioTek).

2.5. Protein isolation and western blot analysis

The cells were seeded in 6-well plates at a density of 5×10^5 (for the 24 h treatment). After exposure, the cells were lysed with 50 μl of ice-cold lysis buffer (50 mM Tris–HCl at pH 7.5, 100 mM NaCl, 0.5% Na-deoxycholate, 0.5% SDS and 0.5% Nonidet P-40) containing protease inhibitors (complete, mini, EDTA-free protease inhibitor cocktail tablet, one tablet per 10 ml lysis buffer, Roche Diagnostic GmbH Mannheim, Germany) and scraped from the wells. The extracts were homogenized by ultrasonication for 10 s in a Bandelin Sonoplus HD 2070 ultrasonic homogenizer (Bandelin Electronic, Berlin, Germany) and centrifuged at 16,000g for 20 min at 4 °C. The total protein concentration of the supernatants was determined with the Bradford reagent (Bio-Rad Protein Assay; Bio-Rad Laboratories, Munchen, Germany) using bovine serum albumin (BSA) as the standard. Protein lysates (40 μg) were mixed with Laemmli sample buffer (BioRad) and boiled for 5 min at 95 °C. The samples were loaded onto a 10% SDS-PAGE gel with a 5% stacking gel and separated in a Bio-Rad Mini-Protean Tetra Cell. Following

Download English Version:

https://daneshyari.com/en/article/5861516

Download Persian Version:

https://daneshyari.com/article/5861516

Daneshyari.com