Hydroxy and methoxy (OH- and CH3O-) derivatives of PBDEs are disruptors of in vitro steroidogenic metabolism

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INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are widely used as additive flame retardants in many different polymers, resins and substrates. Due to the widespread production and use of PBDEs, their high binding affinity to particles, and their lipophilic characteristics, several PBDE congeners (bio)accumulate in the environment [1]. In addition, PBDEs have been detected in various biotic samples such as birds, seals, whales and human samples. [2, 3]. Furthermore, concentrations of PBDEs have been rapidly increasing during the last 10 years in human breast milk from European and American women [4] and a number of (in vitro) endocrine effects have been reported [5-7]. PBDEs have been shown to be susceptible to several metabolic processes including oxidative and reductive debromination, oxidative CYP enzyme-mediated biotransformation, and/or phase II conjugation in the in vivo situation [8]. Hydroxy- and methoxylated BDEs have recently been reported to be present in various biotic samples including herring, salmon and seal [9]. Some in vitro studies have indicated that hydroxylated PBDEs are potential endocrine disruptors (e.g. HO-PBDEs and brominated bisphenol A-analogs, are agonists of both ER alpha and ER beta receptors) [10]. Via the food chain, air and water as well as during fetal development, man and wildlife are exposed to many agents, which can interact with the endocrine system, including these PBDEs and other brominated flame retardants. During the last two years, we focused on environmentally relevant PBDEs including some of their metaboliets and their possible effects on sex hormone synthesis and metabolism.

The human adrenocortical carcinoma cell line (H295R) was used to determine any potencialeffets of PBDEs on two key enzyme activities in the production of sex hormones, CYP19 and CYP17. CYP19 or aromatase mediates the conversion of androgens to estrogens, while CYP17 is responsible for the biosynthesis of weak androgens (e.g. DHEA).

From eighteen different PBDEs tested, none of these congeners showed a significant inductive or inhibitory effect on aromatase or CYP17 activities when cells were exposed to compounds in a concentration range 2.5 up to 7.5 µM[11, 12]. However, some OH BDE derivates decreased CYP19, CYP17 activities in the human H295R cells significantly at concentrations between 2.5 up to 7.5 µM. However, replacing the hydroxy group by a methoxy group decreased this inhibitory effect on CYP19 and CYP17.

In the present study, human placental microsomes have been used to study the possible interaction of 24 hydroxy and methoxy BDE derivates with aromatase. Human placenta is considered to be one of the best in vitro models to study effects on CYP19 activity because of its high production of estrogens involved in gestation of the fetus.

MATERIALS and METHODS

Cell culture conditions

H295R cells were obtained from the American Type Culture Collection (ATCC #CRL-2128) and grown in culture under conditions published previously (Sanderson et al., 2000). Wells (24-well plates) were seeded with 1 ml of cell suspension per well. The culture medium was changed 24h after seeding, during which time the cells attached to the plate and reached almost confluence, after which the cells were exposed to the test chemicals. These were added to the wells at various concentrations using 1 µl of stock solutions dissolved in DMSO.

Chemicals

In this study, cells were exposed to the following hydroxy and methoxy polybrominated diphenyl ethers (OH-BDE sand CH_3O-BDEs): 6'-OH-BDE49, 6'-MeOBDE49, 3-MeO-BDE47, 3-OH-BDE47, 6-MeO-BDE90, 4'-OH-BDE49, 4'-MeO-BDE49, 2'-OH-BDE28, 4-OH-BDE42, 4-MeO-BDE42, 2'-OH-BDE68, 2'-MeO-BDE68, 6-OH-BDE47, 6-MeO-BDE47, 5-OH-BDE47, 5-MeO-BDE47, 4'-OH-BDE17, 4'-MeOBDE17, 2'-OH-BDE66, 2'-MeO-BDE66. The compounds were synthesized by Dr. GoranMarshs at the Wallenberg Laboratory (Stockholm University, Sweden). Concentrations tested ranged from 0.01mM up to 100mM.

Aromatase assay

The catalytic activity of aromatase was determined based on the tritiated water-release method of Lephart and Simpson (1991). The specificity of the aromatase assay based on the release of tritiated water was verified by measuring the production of estrone that is the aromatization product of androstenedione, using a 125I-labeled double-antibody radioimmunoassay kit (DSL-8700; ICN,Costa Mesa, CA), and by using 4-HA, an irreversible inhibitor of the catalytic activity of aromatase, to block the formation of tritiated water from 1β-3H-androstenedione (Sanderson et al., 2001).

Enzymatic activity (CYP17) assay

After addition of 0.1µM Pregnenolone (precursor) the catalytic activity of CYP17 was determined in exposed H295R cells based on the production of its product dehydroepiandrosterone (DHEA), which was measured using a RIA kit (Radioimmunoassay #IM1138, Immunotech, Bechman Coulter Company). In order to measure CYP17 activity without interference of pregnenolone metabolism into mineral and glucocorticoids, the enzyme 3β-hydroxysteroid dehydrogenase was simultaneously blocked with Trilostane (1 µM). SU 10863 (1µM) was used as a positive control for CYP17 inhibition

Human placenta microsome fraction

A human placenta was given by Dr. Paul de Jong (Antonius Hospital (Niewengein, The Netherlands)) and stored at –70˚C. In order to isolate the microsomal fraction from the human tissue, the samples were weighed and homogenized in 10 volumes of TRIS-HCl buffer by using a plotter device. There after the tubes were centrifuged for 25 minutes at 15,000 rpm at 4°C. The supernatant was pipetted into a clean ultra-centrifuge tube and centrifuged for 1:15hr at 47,000 rpm at 4°C. Then, the supernatant was decanted and the pellet resuspended in sucrose solution (0.25M). After that 3 ml of suspension in tubes was taken with 147 ml milliQ for protein measurement and the microsome suspension was frozen in aliquots at -70 °C and stored until use.

RESULTS and DISCUSSION

H295 cell line. Initial range finding experiments were done with the H295R cell line and five different OH-BDEs showing significant inhibition of CYP19 activity after 24h. An decrease of 50% and 90% of the control aromatase activity was found at 7.5 µM for 6OH-BDE99 and 6OH-BDE47,respectively (Fig.1a). However, in the case of 6OH-BDE47 the effects on aromatase were partly due to high cytotoxicity (data not shown)

Similar results were found for CYP17 activity when the H295R cell line was exposed to 5 different OH BDEs. CYP17 activity was significantly inhibited when the cells were exposed to 6OH-BDE47, 6OH-BDE99 or 4OH-BDE49 at 10 µM (Fig.1b).

Substitution of the OH group by a CH3O group eliminated the inhibitory effects on CYP19 and CYP17. Only in the case of 6CH3O-BDE47 a significant aromatase and CYP17 inhibition remained (Fig.2), which was not caused by cytotoxicity as was the case for the OH analogue (data not shown).

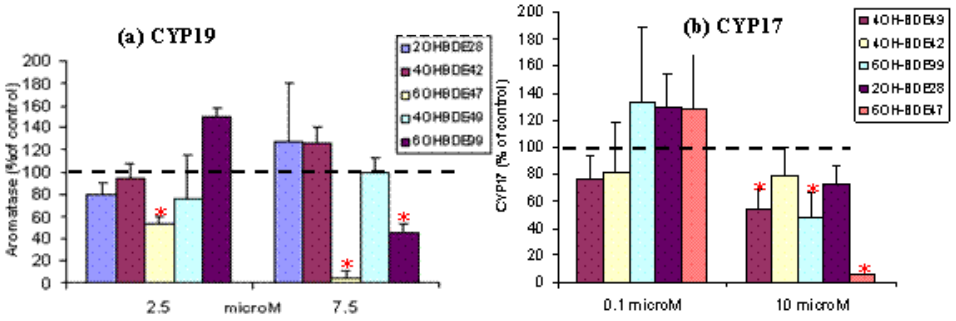

Figure 1. Effects of 5 different OH-BDEs on CYP19 (a) and CYP17 (b)activity after 24h of exposure in H295R cell line. Values represent means ± standard deviations (SD). (*) Significantly lower from the control (p<0.05)

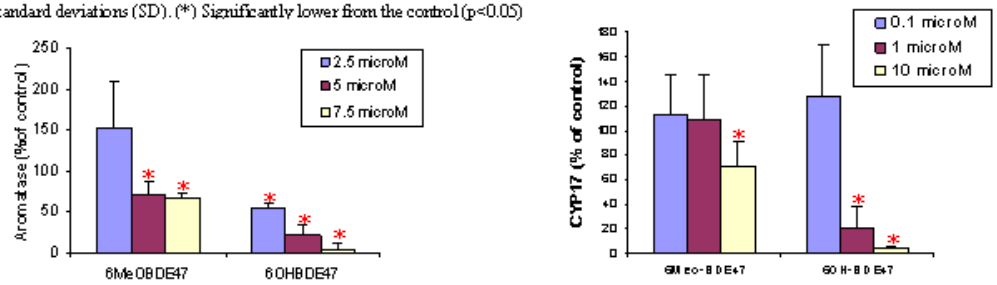

Figure 2. Inhibitory effects on CYP19 and CYP17 activity of 6CH3O-BDE47 still present after replacement of hydroxy group of 6OH-BDE47 by a methoxy group. Values represent means ± standard deviations (SD). (*) Significantly lower from the control (p<0.05)

Placental microsomes

A selection of 24 OH and MeO derivates of PBDEs was used to study possible inhibitory properties on aromatase activity in human placental microsomes. All hydroxy-BDE derivates showed significant inhibition of placental aromatase in the concentration range 0.01 to 10 µM (results of some of the OH-BDEs are included in Fig.3). However, methoxy analogues of these hydroxy derivates were also tested and none of these showed a significant effect on aromatase within this concentration range (results of some of the MeO-BDEs are included in Fig.3). It should be noted that in these `ex vivo` experiments with human placenta the observed inhibition could only be caused by direct interaction of these compounds with the aromatase enzyme and not be a result of cytotoxicity, as was the case in the experiments with H295R cells. The results of our experiments with the human adrenocarcinoma H295R cells and the placental micromes provide a strong indication that OH and MeO BDEs can act as disruptors in different steps of the steroid synthesis and metabolism. Our experiments with placental microsomes furthermore indicate that catalytic inhibition is the most likely cost for the observed effects.

Further studies are necessary to establish if these inhibitions of aromatase and CYP17 by OH BDEs also occur in vivo and have relevance for the human exposure situation.

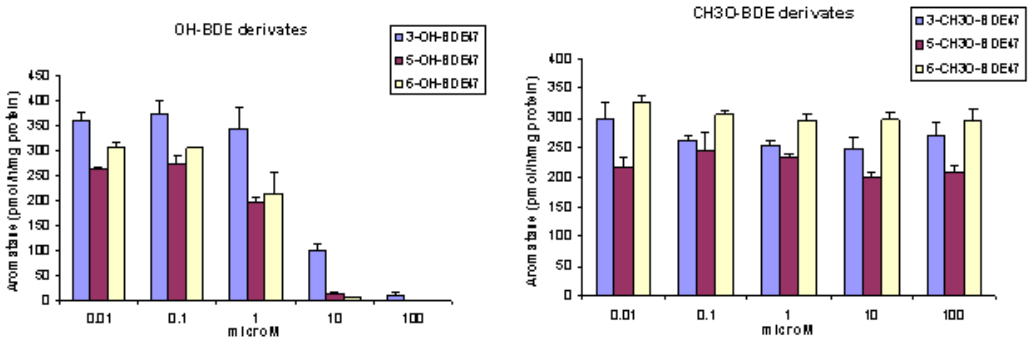

Figure 3. Effects of several OH and MeO-BDEs on CYP19 and CYP17 activity after exposure in human placental microsomes. Values represent means ± standard deviations (SD).

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