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In vitro effects of brominated flame retardants and metabolites on CYP17 catalytic activity: A novel mechanism of action?

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Abstract

Fire incidents have decreased significantly over the last 20 years due, in part, to regulations requiring addition of flame retardants (FRs) to consumer products. Five major classes of brominated flame retardants (BFRs) are hexabromocyclododecane isomers (HBCDs), tetrabromobisphenol-A (TBBPA) and three commercial mixtures of penta-, octa- and deca-polybrominated diphenyl ether (PBDE) congeners, which are used extensively as commercial FR additives. Furthermore, concentrations of PBDEs have been rapidly increasing during the 1999s in human breast milk and a number of endocrine effects have been reported. We used the H295R human adrenocortical carcinoma cell line to assess possible effects of some of these BFRs (PBDEs and several of their hydroxylated (OH) and methoxylated (CH_3O) metabolites or analogues), TBBPA and brominated phenols (BPs) on the combined 17α-hydroxylase and 17,20-lyase activities of CYP17. CYP17 enzyme catalyzes an important step in sex steroidogenesis and is responsible for the biosynthesis of dehydroepiandrosterone (DHEA) and androstenedione in the adrenals. In order to study possible interactions with BFRs, a novel enzymatic method was developed. The precursor substrate of CYP17, pregnenolone, was added to control and exposed H295R cells, and enzymatic production of DHEA was measured using a radioimmunoassay. In order to avoid pregnenolone metabolism via different pathways, specific chemical inhibitor compounds were used. None of the parent/precursor BFRs had a significant effect ($P < 0.05$) on CYP17 activity except for BDE-183, which showed significant inhibition of CYP17 activity at the highest concentration tested (10 μM), with no signs of cytotoxicity as measured by mitochondrial toxicity tests (MTT). A strong inhibition of CYP17 activity was found for 6-OH-2,2′,4,4′-tetrabromoDE (6-OH-BDE47) with a concentration-dependent decrease of almost 90% at 10 μM, but with a concurrent decrease in cell viability at the higher concentrations. Replacement of the 6-OH group by a 6-CH_3O group eliminated this cytotoxic effect, but CYP17 activity measured as DHEA production was still significantly inhibited. Other OH- or CH_3O-PBDE analogues were used to elucidate possible structural properties behind this CYP17 inhibition and associated cytotoxicity, but no distinct structure activity relationship could be determined.

These in vitro results indicate that OH and CH_3O-PBDEs have potential to interfere with CYP17 activity for which the in vivo relevance still has to be adequately determined.

Keywords: Brominated flame retardants (BFRs); Polybrominated diphenyl ethers (PBDEs); Hydroxylated PBDEs (OH-PBDEs); Methoxylated PBDEs (CH_3O-PBDEs); CYP17; Dehydroepiandrosterone (DHEA); H295R human adrenocortical carcinoma cells

Introduction

Brominated flame retardants (BFRs) are used, among others, in plastics, electronic equipment, television sets, mobile devises, building materials and textiles to increase their resistance to fire. BFRs have become an increasingly important group of organohalogen compounds, which include hexabromocyclododecane isomers (HBCDs), tetrabromobisphenol-A (TBBPA) and commercial mixtures of penta-, octa- and deca-brominated diphenyl ether (PBDE) congeners, which are extensively used at high production–volume levels (e.g., in

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2001, the total market demand for PBDEs was 67,000 tonnes including 7500 tonnes of the penta-BDE product; Kierkegaard et al., 2004). Nowadays, penta- and octa-BDE mixtures have been banned in Europe and production voluntarily stopped in United States.

Some BFR compounds were found in environmental samples as long as 25 years ago, but they are now found globally in biota and accumulate in lipid-rich organisms, e.g., fatty fish and marine mammals (Law et al., 2003; Hites, 2004). Their physical and chemical properties can explain these increased levels in biotic tissues. Many BFRs are lipophilic and slowly metabolized; they also have high binding affinity to organic matter and a tendency to accumulate in sediment (Darnerud et al., 2001; Alaee et al., 2003).

In vitro studies have shown that certain BFRs can affect thyroid hormone homeostasis by acting as potent competitors of T_4 for binding to human transthyretin and thyroid hormone receptors (Meerts et al., 2000; Zhou et al., 2002). Some PBDEs have also shown interactions with the estrogen receptor in vitro by stimulating an ER-mediated luciferase reporter gene (Meerts et al., 2001; Olsen et al., 2002). In addition, European concentrations of PBDEs in human milk have increased during the period 1972–1997, from 0.07 to 4.02 ng/g lipid weight, indicating a doubling in levels every 5 years (Noren and Meironyte, 2000). Recent studies have shown that PBDE levels seem to have reached a maximum value and are starting to decrease slowly (Sellstrom et al., 2003). This is probably related to the European ban on some BFR compounds, e.g., the penta-BDE mixture, during the last years. However, in North America levels are on average 10–20 times higher compared to the European situation.

In exposed organisms, including humans, toxic effects, bioaccumulation, metabolism and pharmacokinetics are important criteria for the risk assessment of BFRs. In rats and mice, tissue distribution and metabolism of several PBDEs (e.g., 2,2′,4,4′-tetrabromoDE (BDE-47), 2,2′,4,4′,5-pentabromoDE (BDE-99) and 2,2′,3,3′,4,4′,5,5′,6,6′-decabromoDE (BDE-209)) and TBBPA have been investigated (Orn and Klasson-Wehler, 1998; Hakk et al., 2002; Darnerud, 2003; Morck et al., 2003; Sandholm et al., 2003; Staskal et al., 2005; Sanders et al., 2006). BDE-47 and -99 have been shown to be rapidly absorbed and distributed among lipid rich tissues. In contrast, TBBPA and BDE-209 were rapidly excreted; resulting from a relatively fast metabolism and/or elimination (Sandholm et al., 2003; Thomas et al., 2005; Thuresson et al., 2005). In the case of BDE-209, biotransformation into hydroxyl (OH-) and methoxylated (CH_3O-) PBDEs or lower brominated biphenyl ethers in combination with a low bioavailability in general from the gastrointestinal tract has been reported in dietary exposure studies with common carp (Stapleton et al., 2004).

OH- and CH_3O-BDEs have also been reported in the blood and liver of wildlife and humans (Haglund et al., 1997; Asplund et al., 1999; Hovander et al., 2002; Hakk and Letcher, 2003; Valters et al., 2004). However, the origin of these derivatives remains controversial. Some of these OH-PBDEs (e.g., 6-OH-BDE47) can be produced by marine organisms such as sponges or ascidians, but in higher vertebrate species P450 enzyme-mediated processes can also produce these OH-PBDEs. Moreover, HBCD isomers, 2,4,6-tribromophenol (TBP) and TBBPA, have found to be toxic to aquatic organisms and may have long-term adverse effects in the aquatic environment (Gribble, 1996; de Wit, 2002; Ronisz et al., 2004).

Consequently, the concern about BFRs and their derivatives with respect to their potential as endocrine disruptors has been growing in humans and wildlife during the last decade. Potential endocrine disruptor compounds may cause reproductive problems, certain cancers and other toxicities related to (sexual) differentiation, growth and development if present at sufficiently high levels. Several cytochrome P450 (CYP) enzymes are responsible for the highly specific reactions in the steroid biosynthesis pathway and are potential targets for endocrine disruption. These steroidogenic enzymes are responsible for the biosynthesis of various steroid hormones, including glucocorticoids, mineralocorticoids, progestins and sex hormones (estrogens and androgens). Androgens and subsequently estrogens are ultimately derived from cholesterol via the formation of pregnenolone, 17-alpha-hydroxypregnenolone and DHEA, the latter two synthesized by CYP17. Androgens may subsequently be converted to estrogens by the enzyme aromatase (CYP19). Thus, both CYP17 and CYP19 catalyze key steps in the production of sex hormones in humans.

The CYP17 enzyme catalyzes two different enzymatic steps, steroid 17α-hydroxylase and 17,20-lyase activities, and is responsible for the production of DHEA, which is synthesized abundantly in the adrenal gland in humans (Chen and Parker, 2004). The plasma levels of DHEA rise continually from the age of 6 to 7, reaching a maximum in the second decade of life, after which it declines to about 15% of the peak level in the ninth decade of life.

Both in vitro and in vivo experimental studies strongly indicate that DHEA is related to anti-obesity, anti-tumor, anti-aging and anti-cancer effects (Ciolino et al., 2003). DHEA is a potent non-competitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH) and as a consequence lowers NADPH levels and reduces NADPH-dependent oxygen-free radical production. Furthermore, in rats, DHEA inhibited the expression of some hepatic carcinogen-activating enzymes like CYP1A1 and CYP1A2 (Labrie et al., 2003; Schwartz and Pashko, 2004).

Recent studies in our laboratory focused on potential interactions of a wide range of BFRs with sex hormone synthesis and metabolism. Previous results from our research group showed inhibitory and inductive effects by certain BFRs in the H295R human adrenocortical carcinoma cell line on aromatase (CYP19) activity. H295R cells express a large number of steroidogenic enzymes (Gazdar et al., 1990) and were also used in the present study, to develop a new enzymatic method for CYP17 activity measurement and to assess possible effects of selected BFRs and their metabolites.