Environmental Toxicology and Pharmacology 58 (2018) 196–201

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Hydroxylated and sulfated metabolites of commonly occurring airborne polychlorinated biphenyls inhibit human steroid sulfotransferases SULT1E1 and SULT2A1

Victoria S. Parker[a], Edwin J. Squirewell[a], Hans-Joachim Lehmler[b], Larry W. Robertson[b], Michael W. Duffel[a,*]

[a] Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, Iowa City, IA, United States
[b] Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, Iowa City, IA, United States

ARTICLE INFO

Keywords:
Sulfotransferase
Estradiol
Dehydroepiandrosterone
Polychlorinated biphenyls
Hydroxylated polychlorinated biphenyls
SULT1E1
SULT2A1
Endocrine disruption

ABSTRACT

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants that are associated with varied adverse health effects. Lower chlorinated PCBs are prevalent in indoor and outdoor air and can be metabolized to their hydroxylated derivatives (OH-PCBs) followed by sulfation to form PCB sulfates. Sulfation is also a means of signal termination for steroid hormones. The human estrogen sulfotransferase (SULT1E1) and alcohol/hydroxysteroid sulfotransferase (SULT2A1) catalyze the formation of steroid sulfates that are inactive at steroid hormone receptors. We investigated the inhibition of SULT1E1 (IC50s ranging from 7.2 nM to greater than 10 μM) and SULT2A1 (IC50s from 1.3 μM to over 100 μM) by five lower-chlorinated OH-PCBs and their corresponding PCB sulfates relevant to airborne PCB-exposure. Several congeners of lower chlorinated OH-PCBs relevant to airborne PCB exposures were potent inhibitors of SULT1E1 and SULT2A1 and thus have the potential to disrupt regulation of intracellular concentrations of the receptor-active steroid substrates for these enzymes.

1. Introduction

Polychlorinated biphenyls (PCBs) remain as persistent environmental toxicants that cause numerous adverse health effects (Ampelman et al., 2015; ATSDR, 2000). PCBs were produced in large quantities in the mid-twentieth century for extensive use in electrical transformers, fluorescent light ballasts, caulk, paint, flame retardants, and many other applications (Erickson and Kaley, 2011). In addition to these legacy sources, there are newly recognized sources of exposures to PCBs, and these include their presence as unintentional byproducts in paints and pigments (Hu and Hornbuckle, 2010; Shanahan et al., 2015).

PCBs undergo enzyme-catalyzed oxidative reactions in biological systems to form hydroxylated PCBs (OH-PCBs), and the formation, physical and chemical properties, and toxicities of these metabolites have been recently reviewed (Dhakal et al., 2017; Grimm et al., 2015b). In many cases it is either the OH-PCBs or their subsequent metabolites that are directly involved in the toxicities observed upon exposure to PCBs. For example, metabolism of OH-PCBs to reactive electrophiles is implicated in genotoxic responses to some PCBs (Robertson and Ludewig, 2011). Metabolism of OH-PCBs can also yield the corresponding sulfuric acid esters (PCB sulfates), and these sulfated metabolites bind with high affinity to human serum proteins such as transthyretin and albumin (Grimm et al., 2013; Rodriguez et al., 2016). Displacement of thyroxine by the binding of OH-PCBs and/or PCB sulfates may disrupt thyroid hormone-dependent processes in susceptible tissues (Brouwer et al., 1998; Grimm et al., 2013). In general, binding to serum proteins by PCB sulfates may also serve as a mechanism for transport of PCB sulfates to tissues, where action of sulfatases may convert the molecule back to OH-PCBs, thus setting up a dynamic cycle between the OH-PCB and the PCB-sulfate. Indeed,

Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; OH-PCB, hydroxylated polychlorinated biphenyl; 4′-OH PCB 3, 4-chloro-4′-hydroxybiphenyl; 4-OH-PCB 8, 2,4′-dichloro-4-hydroxybiphenyl; 4-OH-PCB 11, 3,3′-dichloro-4-hydroxybiphenyl; 4′-OH PCB 25, 2,3′,4-trichloro-4′-hydroxybiphenyl; 4-OH PCB 52, 2,2',5,5'-tetrachloro-4-hydroxybiphenyl; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; PCB, polychlorinated biphenyl; PCB 28, 2,4,4′-trichlorobiphenyl; 4′-PCB 3 sulfate, 4-chloro-4′-biphenylsulfate; 4-PCB 8 sulfate, 2,4′-dichloro-4-biphenylsulfate; 4-PCB 11 sulfate, 3,3′-dichloro-4-biphenylsulfate; 4′-PCB 25 sulfate, 2,3′,4-trichloro-4′-biphenylsulfate; 4-PCB 52 sulfate, 2,2',5,5'-tetrachloro-4-biphenylsulfate; SULT, human cytosolic sulfotransferase; SULT1E1, human estrogen sulfotransferase 1E1; SULT2A1, human hydroxysteroid sulfotransferase 2A1

* Corresponding author at: Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, The University of Iowa, 115 South Grand Ave, S325, Iowa City, IA, 52246, United States.
E-mail address: michael-duffel@uiowa.edu (M.W. Duffel).

https://doi.org/10.1016/j.etap.2018.01.010
Received 23 January 2018; Accepted 24 January 2018
Available online 03 February 2018
1382-6689/

studies in rats indicate that intravenous injection of 4-PCB 11 sulfate results in rapid uptake by tissues and conversion to phenolic metabolites (Grimm et al., 2015a).

The mammalian sulfation of OH-PCBs is catalyzed by cytosolic sulfotransferases (SULTs). SULTs catalyze sulfation of xenobiotics such as OH-PCBs as well as endogenous molecules that include steroid hormones, bile acids, catecholamines, neurotransmitters, and others (Coughtrie, 2016; Duffel, 2010; Gamage et al., 2006; Glatt et al., 2001; James and Ambadapadi, 2013). For steroid hormones, SULTs play a key role in signaling processes through the conversion of active steroid hormones to inactive steroid sulfates. Although steroid sulfates are inactive at steroid hormone receptors, they may serve as transport forms for the hormone (Labrie et al., 1995). OH-PCBs interact with SULTs as either substrates or inhibitors, depending upon the specific isoform of the enzyme and the chemical structure of the OH-PCB (Ekuase et al., 2014; Ekuase et al., 2011; Kester et al., 2000; Schuur et al., 1998; Wang and James, 2007; Wang et al., 2005; Wang et al., 2006).

Our studies focus on the human estrogen- and hydroxysteroid- sulfotransferases SULT1E1 and SULT2A1, respectively. SULT1E1 catalyzes the sulfation of estradiol and other phenolic steroids, and SULT2A1 catalyzes the sulfation of hydroxysteroids such as dehydroepiandrosterone and various alcohol-containing androgens (Coughtrie, 2016; Falany et al., 1995b). SULT1E1 has a high affinity and catalytic efficiency for estradiol as substrate, and can thereby be involved in estrogenic signaling through regulation of intracellular concentrations of the active hormone. SULT2A1 catalyzes the formation of dehydroepiandrosterone sulfate (DHEAS) the most abundant circulating steroid in humans. DHEAS serves as a transport form for dehydroepiandrosterone (DHEA) in the serum. Following uptake of DHEAS by tissues and its subsequent hydrolysis catalyzed by steroid sulfatase, DHEA serves as a precursor for both androgens and estrogens (Labrie et al., 1995). Thus, both SULT1E1 and SULT2A1 can play important roles in steroid signaling through regulation of the active forms of the hormones.

OH-PCBs are known to be endocrine disruptors, wherein they exert multiple varied effects on hormone-dependent physiological processes (Brouwer et al., 1998; Grimm et al., 2015b; Meerts et al., 2004; Quinete et al., 2014). Inhibition of SULT1E1 has been demonstrated for several OH-PCBs, and inhibition constants at or below physiological concentrations of estradiol suggest that this may be a basis for physiological estrogenic effects observed with some PCBs and OH-PCBs (Kester et al., 2000; Kester et al., 2002). Likewise, inhibition constants for some OH-PCBs as inhibitors of the sulfation of DHEA catalyzed by SULT2A1 are within the range of reported serum concentrations for this steroid hormone (Ekuase et al., 2014; Ekuase et al., 2011).

A less-often studied group of PCBs are those congeners containing fewer than five chlorine atoms. These lower-chlorinated PCBs are highly represented in both indoor and outdoor air samples (Ampleman et al., 2015; Grimm et al., 2015b; Hu et al., 2010), and there is increasing concern about airborne PCB exposures from indoor air of older buildings, in particular within U.S. schools (Herrick et al., 2004; Herrick et al., 2011). We have directed our attention toward inhibition of SULT1E1 and SULT2A1 by OH-PCB metabolites that would likely be derived from human exposure to PCBs in air. Thus, we have examined several para hydroxylated OH-PCBs that would be derived from PCBs 3, 8, 11, and 52 (congeners that are among the ten most commonly observed PCBs in air samples Grimm et al., 2015b). In addition, we have studied 4′-OH-PCB 25, which would be a metabolite of PCB 28, another congener among the ten most commonly encountered PCBs in air. It is noteworthy that 4′-OH-PCB 25 has recently been identified as a metabolite in human plasma with an estimated half-life of 6.5 years (Quinete et al., 2017). Since SULTs may be subject to product inhibition by sulfates (Gulcan and Duffel, 2011; James, 2014; Zhang et al., 1998), we have also explored the potential for the PCB sulfates to inhibit the sulfation of estradiol catalyzed by SULT1E1 and the sulfation of DHEA catalyzed by SULT2A1.

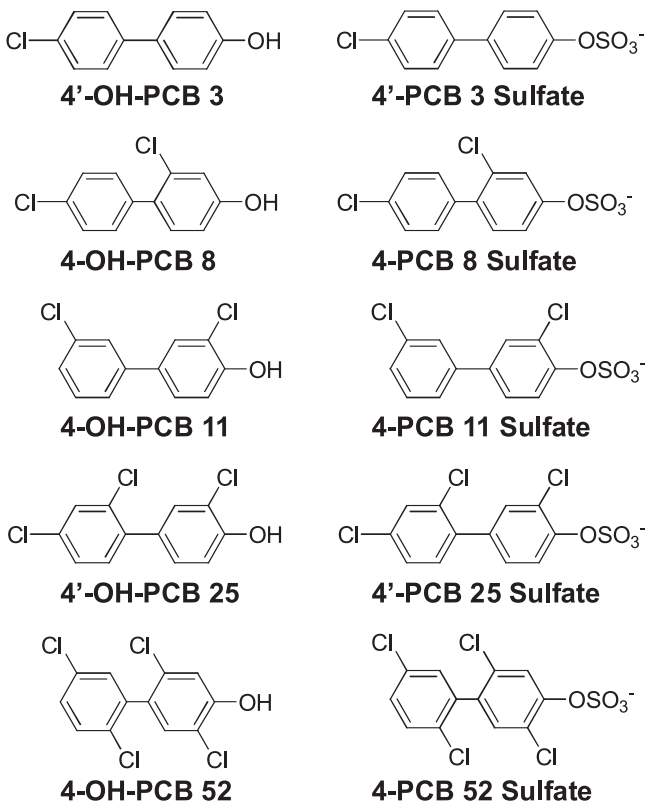

Fig. 1. Structures of PCB metabolites.

2. Materials and methods

2.1. Chemicals and reagents

The synthesis and characterization of each of the PCB metabolites shown in Fig.1 was conducted by the Synthesis Core of the Iowa Superfund Research Program. Briefly, OH-PCBs were synthesized via the Suzuki coupling of a chlorinated benzene boronic acid with a chlorinated iodo- or bromobenzene, followed by demethylation with BBr_3 in dichloromethane as described (Joshi et al., 2011, Lehmler and Robertson 2001, Rodriguez et al., 2016, Zhu et al., 2013). All PCB sulfates were synthesized in two steps by reacting the corresponding OH-PCBs with 2,2,2-trichloroethyl chlorosulfate and, subsequently, removing the 2,2,2-trichloroethyl protective group with zinc powder/ammonium formate (Flor et al., 2015, Grimm et al., 2013, Lehmler et al., 2013, Li et al., 2010, Rodriguez et al., 2016). Adenosine 3′-phosphate 5′-phosphosulfate lithium salt hydrate (PAPS) was purchased from Sigma-Aldrich (St. Louis, MO) and purified as described previously (Sekura, 1981). The purity of the PAPS (99%) was assessed by high performance liquid chromatography using a previously described method (Sheng et al., 2001). [^{3}H]-Dehydroepiadrosterone [1,2,6,7-^{3}H(N)] (49.7 Ci/mmol) and [^{3}H]-estradiol [2,4,6,7- ^{3}H(N)] (81.0 Ci/mmol) were obtained from PerkinElmer (Waltham, MA), and Econo-Safe liquid scintillation cocktail was from Research Products International (Mount Prospect, IL). Absolute ethanol was obtained from Decon Laboratories (King of Prussia, PA). All other chemicals were purchased from ThermoFisher Scientific (Waltham, MA), and were at least ACS grade.

2.2. Expression and purification of SULT1E1 and SULT2A1

Recombinant human SULT1E1 (Squirewell and Duffel, 2015) and SULT2A1 (Gulcan et al., 2008) were expressed in *Escherichia coli* BL21 (DE3) cells, purified, and characterized as described previously.