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Hydroxylated and sulfated metabolites of commonly observed airborne polychlorinated biphenyls display selective uptake and toxicity in N27, SH-SY5Y, and HepG2 cells

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ABSTRACT

Although neurotoxicity and hepatotoxicity have long been associated with exposure to polychlorinated biphenyls (PCBs), less is known about the selective toxicity of those hydroxylated PCBs (OH-PCBs) and PCB sulfates that are metabolites derived from exposure to PCBs found in indoor air. We have examined the toxicity of OH-PCBs and PCB sulfates derived from PCBs 3, 8, 11, and 52 in two neural cell lines (N27 and SH-SY5Y) and an hepatic cell line (HepG2). With the exception of a similar toxicity seen for N27 cells exposed to either OH-PCB 52 or PCB 52 sulfate, these OH-PCBs were more toxic to all three cell-types than their corresponding PCB or PCB sulfate congeners. Differences in the distribution of individual OH-PCB and PCB sulfate congeners between the cells and media, and the ability of cells to interconvert PCB sulfates and OH-PCBs, were important components of cellular sensitivity to these toxicants.

1. Introduction

The man-made environmental contaminants known as polychlorinated biphenyls (PCBs) continue to persist in the environment and cause or contribute to various harmful health effects including neurotoxicity (Schantz et al., 2003; Tilson and Kodavanti, 1997, 1998) and hepatotoxicity (Cave et al., 2010). Although global levels of PCBs have decreased since their production and use have been limited worldwide (Nyberg et al., 2014), the lower-chlorinated PCBs (i.e., ≤4 chlorine atoms per congener) are found in environmental samples (Basu et al., 2009; Hu et al., 2011; Rodenburg et al., 2010) including indoor and outdoor air from both urban and rural areas (Egsmose et al., 2016; Pedersen et al., 2016) and as unintended byproducts from current production of consumer products such as pigments and dyes (Hu and Hornbuckle, 2010; Shang et al., 2014; Vorkamp, 2016). Exposure to these semi-volatile compounds has been proposed to occur through multiple pathways that include inhalation (Hu et al., 2014) as well as the consumption of contaminated food or water (Ampleman et al., 2015; Chen et al., 2017).

Lower-chlorinated PCBs are highly susceptible to metabolic transformation and have often been considered transient species in the body. This metabolic vulnerability, however, also carries with it the potential for production of reactive and toxic compounds (Grimm et al., 2015b; Hansen, 2001; Sethi et al., 2017). Hydroxylated PCB metabolites (OH-PCBs) have been detected in human blood samples and in biological samples from various species (Gutleb et al., 2010; Koh et al., 2016; Marek et al., 2014; Schafer et al., 2009). The oxidation of PCBs to hydroxylated metabolites allows for further metabolism, of which sulfation represents a potentially important route (Grimm et al., 2015b). Although sulfation of a phenolic compound is traditionally considered a mode for its removal from the body due to increased polarity, water solubility, and excretion of the sulfated product, the potential for

Abbreviations: DMEM, Dulbecco's modified eagle's medium; DPBS, Dulbecco's phosphate buffered saline; HS, horse serum; HSA, human serum albumin; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 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 52, 2,2′,5, 5′-tetrachloro-4-hydroxybiphenyl; PCB, polychlorinated biphenyl; PCB 3, 4-chlorobiphenyl; PCB 8, 2,4′-dichlorobiphenyl; PCB 11, 3,3′-dichlorobiphenyl; PCB 28, 2,4,4′-trichlorobiphenyl; PCB 52, 2,2′,5,5′-tetrachlorobiphenyl; 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 52 sulfate, 2,2′,5, 5′-tetrachloro-4-biphenylsulfate; RPMI medium, Roswell Park Memorial Institute medium

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biological retention may also exist. A study in Sprague-Dawley rats has indicated that hydroxylation followed by sulfation accounts for more than half of the metabolic fate after exposure to the monochlorinated PCB 3 (Dhakal et al., 2012). Additional studies in rats following administration of 4-PCB 11 sulfate indicated that some PCB sulfates, however, may be retained in vivo (Grimm et al., 2015a). Furthermore, the presence of 4-PCB 11 sulfate in human serum samples has recently been reported (Grimm et al., 2017). In vitro studies have shown that, while OH-PCBs can inhibit the sulfation of endogenous molecules including dehydroepiandrosterone (DHEA) and estradiol, many OH-PCBs also serve as substrates for sulfate conjugation (Ekuase et al., 2011; Kester et al., 2002; Liu et al., 2006; Parker et al., 2018). The resulting PCB sulfates bind to the thyroid hormone carrying protein transthyretin, where, in some cases, they bind with similar affinity to that observed with thyroxine (Grimm et al., 2013). Moreover, PCB sulfates have been shown to bind with high affinity to the major drug binding sites of human serum albumin (HSA), the most abundant protein in human plasma (Rodriguez et al., 2016). In the case of both transthyretin and HSA, protein binding was influenced by the degree of chlorination and the substitution patterns of the PCB congeners, and PCB sulfates generally bound with a higher or equal affinity than the corresponding PCB or OH-PCB, thereby potentially increasing their retention and distribution in the body. These studies suggest that, contrary to the general assumption that sulfation of a xenobiotic is simply a mode for its excretion, the sulfates derived from lower-chlorinated OH-PCBs may be retained, transported, and have distinct biological and/or toxicological activities.

While little is known about the in vivo toxic effects of PCB metabolites, the neurotoxic and hepatotoxic effects of various PCB mixtures and individual congeners have been well documented in the scientific literature. Exposure to PCBs has been associated with non-alcoholic fatty liver (Cave et al., 2010), and PCBs have been identified as promoting and initiating agents in hepatic carcinogenesis (Ludewig et al., 2008). Epidemiological studies on the neurotoxic effects of PCB exposure indicate correlations with neurodevelopmental dysfunction and with incidences of neurodegenerative diseases (Hatcher-Martin et al., 2012; Steenland et al., 2006). Environmental PCB exposure-related effects on mood, depression, social and reproductive behaviors, cognition and motor function have also been reported (Berghuis et al., 2015, 2013; Jurewicz et al., 2013; Polanska et al., 2013). In vitro studies using cultured neuronal cells have often focused on the cytotoxic effects of higher-chlorinated PCB congeners and Aroclor mixtures (Tilson and Kodavanti, 1997; Tilson et al., 1998). Lower-chlorinated PCBs are, however, of increasing interest, as seen in the recent study of the effect of PCB 11 and its hydroxylated and sulfated metabolites on axonal and dendritic growth in cultured primary rat neuronal cells (Sethi et al., 2017).

We hypothesized that OH-PCB and corresponding PCB sulfate metabolites of lower-chlorinated PCBs exhibit toxicity in cultured cells that is influenced by PCB congener, metabolite, and target cell type. The cell lines used in this study were of neural (rat midbrain N27 and human neuroblastoma SH-SY5Y) and hepatic (human hepatic HepG2) origins. Cellular toxicity was measured using two orthogonal cell viability assays (i.e., the reduction of MTT and the release of lactate dehydrogenase (LDH)). The PCBs and PCB metabolites included in this study (Fig. 1) were chosen to represent some of the most frequently detected PCB congeners in air samples and encompass varying degrees of chlorination and substitution patterns (Grimm et al., 2015b). Moreover, since the presence of *ortho*-substituted chlorines among PCB congeners can dictate their three dimensional structure by influencing the dihedral angle between the phenyl rings, with significant influences on their biological effects (Van den Berg et al., 2006), we have examined both *ortho*-substituted and non *ortho*-substituted congeners. The fate of these molecules in vitro was monitored by HPLC to determine their distribution between cells and extracellular medium. Finally, to determine the effects of albumin-binding on cytotoxicity, studies were performed with HSA supplementation in the incubation media.

The studies presented here probe the roles that metabolism of lower-chlorinated PCBs, particularly hydroxylation and subsequent sulfation, may play in the toxic effects of certain PCB congeners. These changes impart complex differences regarding toxicity profiles, distribution of the metabolites into cells from different tissues, as well as their potential for further metabolic reactions within those cells that influence toxic outcomes.

2. Materials and methods

Cell culture media and media components that were obtained from Gibco (Life Technologies, Madison, WI, USA) included: Roswell Park Memorial Institute (RPMI) medium, Dulbecco's Modified Eagle's Medium (DMEM), Opti-MEM, Dulbecco's phosphate buffered saline (DPBS), Trypsin –EDTA (0.25%), penicillin/streptomycin, sodium pyruvate (100 mM), fetal bovine serum (FBS), horse serum (HS), and MEM non-essential amino acids (MEM NEAA). Corning Falcon tissue culture 100 mm^2 petri dishes, Corning Costar 96-well plates, and dimethyl sulfoxide (DMSO, ≥99.9%) were purchased from Fisher Scientific (Radnor, PA, USA). Collagen Type I, rat tail, was purchased from BD Biosciences (San Jose, CA, USA). Human serum albumin (HSA, fatty acid and globulin free, ≥99%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 98%), and NADH (≥99% by HPLC) were purchased from Sigma Aldrich (St. Louis, MO, USA). Authenticity of the human cell lines was confirmed by analysis of genomic DNA conducted by the University of Arizona Genetics Core (Arizona Research Laboratories, Tucson, AZ). Cell culture and incubations were performed under the standard conditions of 37 °C in a 5% CO_2 atmosphere. All PCBs and their corresponding hydroxylated and sulfated metabolites were prepared and characterized as previously described (Lehmler et al., 2013; Lehmler and Robertson, 2001; Li et al., 2010; Rodriguez et al., 2016). Spectroscopic analyses were performed using a Spectramax M5 fluorimeter (Molecular Devices, Sunnyvale, CA, USA), and statistical analyses and sigmoidal dose response cytotoxicity analyses were obtained using SigmaPlot v.11.0, Systat Software (Chicago, IL, USA).

2.1. N27 cells

Rat midbrain-derived immortalized N27 cells were a generous gift from Dr. Jau-Shyong Hong, Neuropharmacology Group, National Institute of Environmental Health Sciences. N27 cells were seeded at a density of 1×10^6 cells in collagen-coated 100 mm^2 tissue culture dishes and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated HS, penicillin (100 I.U./mL) and streptomycin (100 µg/mL). Medium was changed every other day until the cells were near confluence (approximately four days). The N27 cells used in this study were between passages 17 through 30.

2.2. SH-SY5Y cells

The human neuroblastoma-derived SH-SY5Y cells were grown in collagen-coated 100 mm^2 tissue culture dishes and maintained in Opti-Mem medium supplemented with 10% heat-inactivated FBS, non-essential amino acids, sodium pyruvate (500 µM), penicillin (100 I.U./mL), and streptomycin (100 µg/mL). Medium was changed every other day until the cells were near confluence (approximately seven days). The SH-SY5Y cells used in this study were between passages 15 through 30.

2.3. HepG2 cells

The immortalized human liver-derived HepG2 cells were provided by Ms. Susanne Flor of the University of Iowa Superfund Research Center. HepG2 cells were grown in 100 mm^2 tissue culture dishes and