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Subacute nicotine co-exposure has no effect on 2,2′,3,5′,6-pentachlorobiphenyl disposition but alters hepatic cytochrome P450 expression in the male rat

Marianna Stamou[a,1], Eric Uwimana[b,1], Brenna M. Flannery[a], Izabela Kania-Korwel[b], Hans-Joachim Lehmler[b], Pamela J. Lein[a,*]

[a] Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA
[b] Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, Iowa City, IA, USA

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ABSTRACT

Polychlorinated biphenyls (PCBs) are metabolized by cytochrome P450 2B enzymes (CYP2B) and nicotine is reported to alter CYP2B activity in the brain and liver. To test the hypothesis that nicotine influences PCB disposition, 2,2′,3,5′,6-pentachlorobiphenyl (PCB 95) and its metabolites were quantified in tissues of adult male Wistar rats exposed to PCB 95 (6 mg/kg/d, p.o.) in the absence or presence of nicotine (1.0 mg/kg/d of the tartrate salt, s.c.) for 7 consecutive days. PCB 95 was enantioselectively metabolized to hydroxylated (OH-) PCB metabolites, resulting in a pronounced enrichment of E_1-PCB 95 in all tissues investigated. OH-PCBs were detected in blood and liver tissue, but were below the detection limit in adipose, brain and muscle tissues. Co-exposure to nicotine did not change PCB 95 disposition. CYP2B1 mRNA and CYP2B protein were not detected in brain tissues but were detected in liver. Co-exposure to nicotine and PCB 95 increased hepatic CYP2B1 mRNA but did not change CYP2B protein levels relative to vehicle control animals. However, hepatic CYP2B protein in animals co-exposed to PCB 95 and nicotine were reduced compared to animals that received only nicotine. Quantification of CYP2B3, CYP3A2 and CYP1A2 mRNA identified significant effects of nicotine and PCB 95 co-exposure on hepatic CYP3A2 and hippocampal CYP1A2 transcripts. Our findings suggest that nicotine co-exposure does not significantly influence PCB 95 disposition in the rat. However, these studies suggest a novel influence of PCB 95 and nicotine co-exposure on hepatic cytochrome P450 (P450) expression that may warrant further attention due to the increasing use of e-cigarettes and related products.

1. Introduction

Nicotine is a highly addictive component of cigarette smoke, e-cigarettes and other tobacco products (Yamin et al., 2010; Noel et al., 2011; Regan et al., 2013) that rapidly crosses the blood–brain barrier to upregulate cytochrome P450 enzymes (Anandatheerthavarada et al., 1993a; Miksys et al., 2000). CYP2B6 is upregulated in the brain of human smokers (Miksys et al., 2003), and nicotine has been reported to increase CYP2B expression in the brain of rats and non-human primates while either reducing or not changing hepatic CYP2B expression (Miksys et al., 2000; Lee et al., 2008). These effects of nicotine on CY2B expression in the brain have been linked to altered *in vivo* pharmacokinetics and pharmacodynamics of the neuroactive compound propofol (Khokhar and Tyndale, 2011).

Human CYP2B6 also metabolizes neurotoxic environmental contaminants including polychlorinated biphenyls (PCBs) (Ariyoshi et al., 1995; Warner et al., 2008), polybrominated diphenyl ethers (PBDEs) (Feo et al., 2012) and organophosphorus pesticides (Crane et al., 2012). CYP2B-mediated metabolism alters the neurotoxic potential of PCBs and PBDEs (Kim et al., 2011; Niknam et al., 2013), and is important in both bioactivation and detoxification of organophosphorus pesticides (Foxenberg et al., 2011). Collectively, these studies suggest that nicotine exposure may influence neurotoxicity by modulating CYP2B activity in the

Abbreviations: C_t, fractional amplification (cycle number at which fluorescence exceeds a user-defined threshold); d, day; b.w., body weight; DE, diatomaceous earth; EF, enantiomeric fraction; qPCR, quantitative (real-time) polymerase chain reaction; PCBs, polychlorinated biphenyls; P450, cytochrome P450.

* Corresponding author at: Department of Molecular Biosciences, UC Davis School of Veterinary Medicine, 1089 Veterinary Medicine Drive, Davis, CA 95616, USA. Fax: +1 530 752 7690.
E-mail address: pjlein@ucdavis.edu (P.J. Lein).

[1] These authors contributed equally to this manuscript.

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brain or liver, thereby altering toxicant levels local to cellular targets.

Here we test the hypothesis that repeated nicotine co-exposure alters the disposition of PCB 95 and its metabolites. PCB 95 was chosen for these studies because it is linked to neurotoxic outcomes in humans and rats (Pessah et al., 2010) and it is metabolized by CYP2B1 in rats (Warner et al., 2008; Lu et al., 2013). Furthermore, PCB 95 is a chiral molecule that exists as two non-superimposable mirror images called enantiomers, and the CYP2B-mediated metabolism of PCB 95 is enantioselective (Lu et al., 2013; Kania-Korwel and Lehmler, 2015). Thus, monitoring the enantiomeric signatures of PCB 95 and its metabolites is expected to provide a sensitive readout for investigating changes in P450 enzyme activities in the brain.

We detected PCB 95 in all tissues investigated, including the cortex and cerebellum, and observed considerable enantiomeric enrichment of the first eluting PCB 95 atropisomer; however, co-exposure to nicotine did not alter PCB 95 disposition in the brain or liver. CYP2B1 mRNA and CYP2B protein were not detected in rat brain, and nicotine treatment had no significant effect on levels of CYP2B1, CYP2B3, CYP3A2 and CYP1A2 mRNA or CYP2B protein in the brain. In contrast, we observed a novel influence of PCB and nicotine co-exposure on the expression of hepatic CYP2B1 and CYP3A2 mRNA.

2. Material and methods

2.1. Chemicals

2,2′,3,5′,6-Pentachlorobiphenyl (PCB 95; 99.7% purity), 2,3,4′,5,6-pentachlorobiphenyl (PCB 117; 99% purity), 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204; 99.9% purity) and 2′,3,3′,4,5,5′-hexachlorobiphenyl-4′-ol (4-159, >99.9% purity) were purchased from AccuStandard (New Haven, CT, USA). 3-Methoxy-2,2′,4,5′,6-pentachlorobiphenyl (3-103), 2,2′,3,5′,6-pentachlorobiphenyl-4-ol (4-95), 2,2′,3,5′,6-pentachlorobiphenyl-4′-ol (4′-95), 2,2′,3,5′,6-pentachlorobiphenyl-5-ol (5-95) and 4,5-dimethoxy-2,2′,3,5′,6-pentachlorobiphenyl (4,5-95) were synthesized at >95% purity as described previously (Kania-Korwel et al., 2008; Joshi et al., 2011). The respective chemical structures and abbreviations are shown in Fig. 1. (−)-Nicotine hydrogen tartrate salt (98% purity; CAS number 65-31-6) was purchased from Sigma–Aldrich (St. Louis, MO, USA). ^{1}H and ^{13}C NMR spectra confirmed that the nicotine hydrogen tartrate salt was highly pure (see Supplemental data, Figs. S1 and S2).

2.2. Animals and treatments

Animals were maintained in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health, and with regard for alleviation of pain and suffering under protocols approved by the UC Davis Institutional Animal Care and Use Committee. Adult male Wistar rats (50–55 days old; 209–270 g; Charles River Laboratories, Hollister, CA) were housed individually in standard plastic cages under controlled environmental conditions (22 ± 2 °C, 40–50% humidity) with a normal 12 h light/dark cycle. Food and water were provided *ad libitum*.

Animals were allowed to acclimate for 48 h after delivery to the UC Davis vivarium and then randomly divided into eight experimental groups with four animals per experimental group (Fig. 2): (i) nicotine in sterile saline (1 mg/kg/d, s.c.) for 7 d plus PCB 95 (6 mg/kg/d, p.o. in peanut butter) for days 1–6; (ii) an equal volume of vehicle (sterile saline, 0.3 ml/d, s.c.) for 7 d plus PCB 95 (6 mg/kg/d, p.o. in peanut butter) for days 1–6; (iii) nicotine in sterile saline (1 mg/kg/d, s.c.) for 7 d; or (iv) vehicle (sterile saline, 0.3 ml/d, s.c.) for 7 d. To assess transient effects of nicotine on CYP2B1 expression in the brain, a subset of rats were injected daily with: (v) nicotine (1 mg/kg/d, s.c.) for 1 d or (vi) 3 d; or (vii) an equal volume of vehicle (sterile saline, 0.3 ml/d, s.c.) for 1 d or (viii) 3 d (Fig. 2).

The dose and route of administration of nicotine was based on previous studies demonstrating that daily s.c. injection with 0.3 mg/kg nicotine free base for 7 consecutive days significantly increased CYP2B1 mRNA levels in the cortex of adult male Wistar rats compared to vehicle controls (Miksys et al., 2000). The tartrate salt of nicotine was administered at 1 mg/kg b.w. (2 μmol/kg b.w.), which translates to a dose of 325 μg/kg of nicotine as the free base (Matta et al., 2007). This dose results in blood nicotine levels in the rat comparable to levels in human smokers following 10 cigarettes (Le Houezec et al., 1993). The PCB 95 dose (6 mg/kg/d, which equals 18.4 μmol/kg/d) was based on our previous studies of PCB 95 enantiomeric disposition in the mouse brain (Kania-Korwel et al., 2012). The route of administration of PCB 95 was previously described (Kania-Korwel et al., 2012), and rats consumed the peanut butter mix within minutes.

Animals were euthanized by carbon dioxide inhalation 4 h after the last nicotine injection (approximately 28 h after the last

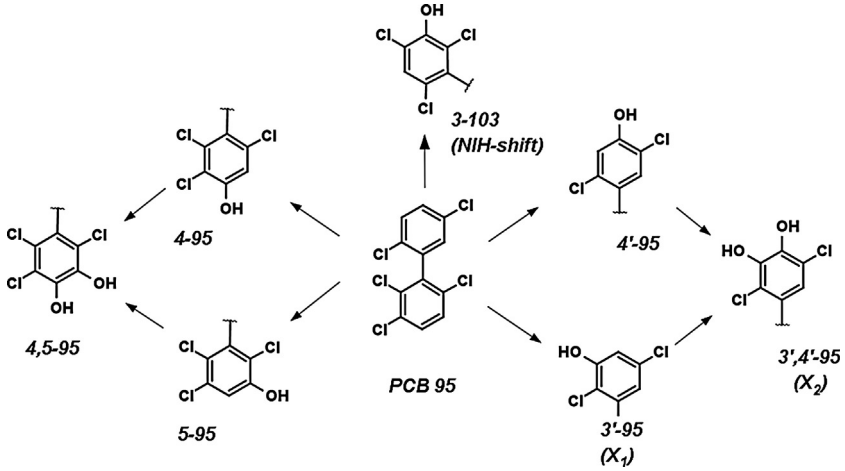

Fig. 1. Simplified scheme of PCB 95 biotransformation showing metabolites unambiguously identified with authentic standards, as well as two unknown metabolites tentatively identified as 3′-95 (X_1) and 3′,4′-95 (X_2).