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Effects of PCB 52 and PCB 77 on cell viability, $[Ca^{2+}]_i$ levels and membrane fluidity in mouse thymocytes

Bayram Yilmaz [a], Suleyman Sandal [a], Chang-Hwei Chen [b,c], David O. Carpenter [b,c,*]

[a] *Firat University, Department of Physiology, Faculty of Medicine, 23119 Elazig, Turkey*
[b] *Institute for Health and the Environment, Department of Environmental Health and Toxicology, University at Albany, SUNY, One University Place, A217, Rensselaer, NY 12144, USA*
[c] *Department of Biomedical Science, School of Public Health, University at Albany, SUNY, Rensselaer NY 12144, USA*

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Abstract

Exposure to polychlorinated biphenyls (PCBs) is known to suppress immune system function and this action is usually ascribed to dioxin-like PCBs that act via the Ah receptor. We have studied the effects of one *ortho*-substituted, non-dioxin-like PCB (PCB 52) and one coplanar, dioxin-like congener (PCB 77) on properties of thymocytes acutely isolated from mice. Viability of thymocytes was dose- and time-dependently reduced by PCB 52 with a threshold concentration of about 1 μM, while there was no effect of PCB 77 on viability at concentrations less than 10 μM. Cell death was detectible within 5 min of exposure. Both congeners caused a dose-dependent increase in $[Ca^{2+}]_i$, but the threshold concentration was 1 μM for PCB 52 and 5 μM for PCB 77. However, the cell death was not due to the elevation of $[Ca^{2+}]_i$, since it was not reduced by incubation in Ca-free Tyrode's Solution. PCB 52, but not PCB 77, caused an increase in membrane fluidity at a concentration of 5 μM. These observations are consistent with previous results that suggest that *ortho*-substituted PCB congeners dissolve in cell membrane and cause greater disruption of function than do dioxin-like PCB congeners.

Keywords: Flow cytometry; Cell death; Immune function; Ah receptor

1. Introduction

Polychlorinated biphenyls (PCBs) are a mixture of up to 209 different congeners having various numbers (1–10) and positions (*ortho*, *meta*, *para*) of chlorine atoms on the biphenyl rings (Apostoli et al., 2003; Carpenter, 1998; WHO, 1993). PCBs were widely used as industrial fluids, flame-retardants, diluents and fluids for capacitors and transformers because of their lipophilic and persistent properties (DeVos et al., 2003). Although their production was banned in most countries in the late 1970s (Carpenter, 1998; Segre et al., 2002; Shin et al., 2000), industrial and household products containing PCB material are still in use.

PCB mixtures are immunotoxic (Silkworth et al., 1984), carcinogenic (Cogliano, 1998), neurotoxic (Chen et al., 1994; Rogan and Gladen, 1992) and cause birth

* Corresponding author. Tel.: +1 518 525 2660; fax: +1 518 525 2665.
E-mail address: carpent@uamail.albany.edu (D.O. Carpenter).

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defects (Safe, 1994) in both animals and humans. However, different PCB congeners have varying patterns of toxicity. Congeners with one or no chlorines in the *ortho* positions (mono-*ortho* and non-*ortho*, respectively) are able to assume a planar configuration, bind to the aryl hydrocarbon (Ah) receptor and have a pattern of toxicity similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Safe, 1994). However, there is also abundant evidence from in vivo and in vitro studies that the *ortho*-substituted, non-coplanar, non-dioxin-like congeners have toxic effects on various organ systems (Hansen, 1999).

The immune system, particularly that of developing animals and humans, is sensitive to chemical insult posed by PCBs, TCDD and polychlorinated dibenzofurans (Van Loeveren et al., 2000). Impaired immune functions have been reported in Taiwanese residents following consumption of rice oil contaminated with PCBs (Chang et al., 1981) and in Yu-Cheng children after a similar poisoning in Japan (Yu et al., 1998). Dutch children exposed prenatally to PCBs and dioxins had more frequent incidences of ear infections and chicken pox than less exposed children (Weisglas-Kuperus et al., 2000).

PCBs have been shown to suppress both humoral and cellular immunity (Silkworth et al., 1984; Smialowicz et al., 1989; Talcott and Koller, 1983; Tryphonas et al., 1989), and it is generally believed that immunosuppressive actions of PCBs are mediated via activation of the Ah receptor (Tryphonas et al., 1991). Coplanar PCB congeners impair immune function by suppressing cytotoxic T lymphocyte activity and antibody production in exposed animals (Kerkvliet et al., 1990a; Silkworth et al., 1984). Rats born and raised by dams fed a continuous diet of Baltic Sea fish oil, which is contaminated with PCBs, showed impaired immune responses as compared to control rats (Ross et al., 1997). However, an Ah-independent mechanism has also been suggested (Kerkvliet et al., 1990a,b; Smithwick et al., 2003). Reduced antibody responses (Davis and Safe, 1990) and superoxide anion production by neutrophils (Ganey et al., 1993) have been attributed to immunotoxic effects of *ortho*-substituted PCB congeners. Harper et al. (1993) showed that splenic plaque-forming cell responses to antigens were reduced by *ortho*-substituted PCB congeners, which are inactive at the Ah receptor. Similarly, lipopolysaccharide-induced splenocyte proliferation was preferentially inhibited by individual congeners with multiple *ortho* substitutions, while suppression of antibody secretion was not attributed by a particular chlorine substitution pattern (Smithwick et al., 2003).

Ortho-substituted PCBs may exert direct cytotoxic effects on immune cells. Yoo et al. (1997) showed that apoptosis was a mechanism of action for PCBs which affected the immunological status of splenocytes. Induction of apoptosis by Aroclor 1254 (a highly chlorinated PCB mixture) as well as PCB congeners 47 (2,2′,4,4′-tetrachlorobiphenyl), 52 (2,2′,5,5′-tetrachlorobiphenyl), 128 (2,2′,3,3′,4,4′-hexachlorobiphenyl) and 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) have been attributed to mechanism(s) independent of Ah receptor activation, since these effects were observed in Ah-receptor knockouts and Ah low-response mice (Jeon et al., 2002).

PCBs are highly lipophilic substances and are stored in body fats, including cellular membranes. Membrane fluidity is determined by the lipid composition of the membranes (Los and Murata, 2004), and substances which alter membrane fluidity also alter physiological function in cells (Woodson et al., 1976). *Ortho*-substituted PCBs are cytotoxic and alter $[Ca^{2+}]_i$ homeostasis in neurons (Kodavanti et al., 1993; Tan et al., 2004; Wong et al., 1997). *Ortho*-substituted PCBs have similar effects on rat thymocytes (Tan et al., 2003), and our previous studies provided evidence that the cytotoxicity and altered calcium homeostasis were secondary to altered membrane fluidity (Tan et al., 2004). In the present study, we have expanded this kind of investigation to mouse thymocytes. We used the non-*ortho* chlorine substituted PCB, coplanar congener (3,3′,4,4′-tetrachlorobiphenyl; IUPAC #PCB 77) and di-*ortho* chlorine substituted, non-coplanar PCB congener (2,2′,5,5′-tetrachlorobiphenyl; IUPAC #PCB 52) in order to compare their effects on cell viability, $[Ca^{2+}]_i$ levels and membrane fluidity in thymocytes from a different species.

2. Materials and methods

2.1. Chemicals and reagents

PCBs 52 and 77 were purchased from Ultra Scientific (North Kingstown, RI). Propidium iodide (PI), 1,6-diphenyl-1,3,5-hexatriene (DPH) and fluo-3 were purchased from Molecular Probes, Inc. (Eugene, OR). Sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) and sodium phosphate dibasic anhydrous (Na_2HPO_4) were purchased from Fisher Scientific (Fair Lawn, NJ). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Bi-distilled water was used in all the experiments. PCBs were dissolved in DMSO such that the final DMSO concentration was never greater than 0.2%. There was no effect of 0.2% DMSO on any of the parameters measured.