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Induction of cytochromes P450, caspase-3 and DNA damage by PCB3 and its hydroxylated metabolites in porcine ovary

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Abstract

Polychlorinated biphenyl (PCBs) levels of tens and hundreds of pg/ml for individual congeners are measured in human follicular fluid. PCB3 (4-chlorobiphenyl), caused a significant increase in estradiol secretion in porcine granulose–theca cell co-cultures and its two metabolites, 4-OH-PCB3 and 3,4-diOH-PCB3, were even more potent than PCB3 itself [Ptak, A., Ludewig, G., Lehmler, H.J., Wojtowicz, A.K., Robertson, L.W., Gregoraszczuk, E.L. 2005. Comparison of the actions of 4-chlorobiphenyl and its hydroxylated metabolites on estradiol secretion by ovarian follicles in primary cells in culture. Reprod. Toxicol. 20, 57–64]. The question is whether these follicle cells are potentially able to metabolize PCB3 to hydroxylated and genotoxic or cytotoxic intermediates. We report here that granulose–theca co-cultures express xenobiotic-metabolizing cytochrome P450 activities, with CYP1A1 > CYP2B ≫ CYP1A2. A significant increase in CYP1A1 and 2B, but not CYP1A2, activity was seen in cells that were exposed to 6 ng/ml PCB3 or 20 nM 17-β-estradiol. An increase in caspase-3 activity, indicative for apoptosis, was only observed in PCB3-exposed cells after 24 h exposure. Genotoxicity, determined with the Comet assay, was initially reduced after 24 h exposure to PCB3 and both metabolites compared to untreated controls, followed by a significant transient increase in Comets at the 4 and 24 h time point with PCB3 and 4-OH-PCB3. 3,4-diOH-PCB3 induced a significant increase only after 72 h of recovery. We hypothesize that these biphasic damage kinetics may be due to cross-links caused by adduct formation. These results show for the first time that granulose–theca cells in co-culture express CYP1A1, 2B and 1A2 activities and that PCBs at concentrations that are reached in the environment induce genotoxicity in granulosa cells.

Keywords: PCB3; Hydroxylated metabolites; Prepubertal pig ovary; CYP; DNA damage; Caspase-3 activity

1. Introduction

Polychlorinated biphenyls (PCBs) are industrial chemicals that were produced as commercial mixtures of chlorinated biphenyls in many countries of the world and sold under various trade names, such as Clophen, Aroclor and Kanechlor, over many decades (Silberhorn

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et al., 1990). PCBs were incorporated into numerous commercial products and have escaped into the environment. Individuals may be exposed to PCBs via their food (a predominant route of exposure for the average citizen), via the skin (e.g. dermal exposure in capacitor workers), or by inhalation (exposure occurring in contaminated buildings, or near waste sites) (ATSDR, 2000).

A number of toxic signs and symptoms have been identified in individuals suffering occupational and accidental PCB exposures (Cogliano, 1998), and the underlying mechanisms of many of these have been investigated in animal studies. A well-characterized response to PCBs is the induction (increased expression) of a broad spectrum of xenobiotic-metabolizing enzymes (Safe et al., 1985; Safe, 1994), including several cytochrome P450 monooxygenases (CYPs). By virtue of interactions with the aryl hydrocarbon receptor (AhR) (Bandiera et al., 1982), the constitutive and rostane receptor (CAR) (Waxman, 1999), and pregnane-X receptor (PXR) (Hurst and Waxman, 2005), PCBs increase the expression of CYPs 1A, 2B and 3A subfamilies, respectively. The number and position of chlorine atoms on the biphenyl ring determine their interaction with these receptors. In general PCBs that function as inducers of CYPs in rodent livers are *meta*-, *para*-, chloro-substituted (CYP1A), *ortho*-, *para*-substituted (CYP2B) and multi-*ortho* substituted (CYP3A) (Denomme et al., 1983; Parkinson et al., 1983; Schuetz et al., 1998).

CYPs have been characterized in the liver, but have also been detected in the extrahepatic tissues such as lung, prostate gland, uterus, adrenal glands, placenta, kidney, brain and testis (Henderson et al., 1992; Hakkola et al., 1996; Lacroix et al., 1997; de Wildt et al., 1999; Raunio et al., 1999; Zhang et al., 1999; Nishimura et al., 2003). The ovaries also contain CYPs. CYP2E1, CYP2A, CYP2B mRNA and protein are expressed in the mouse ovary (Cannady et al., 2003). Pig and human ovaries contain CYP1A1 and CYP1B1 isoforms (Hammond et al., 1986; Hakkola et al., 1997; Muskhelishvili et al., 2001). Leighton et al. (1995) found CYP1A1 mRNA in porcine ovarian granulosa cells and a porcine ovarian granulosa cells line (MDG2.1). For these reasons the ovaries may play an important role in the metabolism of endogenous and exogenous compounds.

PCBs, especially the less chlorinated congeners, may also be substrates for these CYPs themselves, and the metabolism of PCBs produces oxygen-containing metabolites, i.e. PCB-derived phenols, catechols, quinones, sulfones and bound residues (reviewed in Bergman et al., in press). The cytochrome P450 catalyzed oxidation of lower chlorinated biphenyls, especially mono-, di- and tri-chlorobiphenyls, gives rise to monohydroxy and dihydroxy metabolites (McLean et al., 1996a). These dihydroxy compounds may be further oxidized to reactive metabolites which form adducts with nitrogen and sulfur nucleophiles, including DNA (Amaro et al., 1996; Oakley et al., 1996a; Zhao et al., 2004). CYPs may also be responsible for the biosynthesis and metabolism of endogenous compounds such as steroid hormones, fatty acids and prostaglandins (Waxman, 1988; Nelson et al., 1996; Rendic and Di Carlo, 1997; Gonzalez and Kimura, 1999). CYPs are involved in the metabolism of estradiol to catechol estrogens in the liver (Dannan et al., 1986; Suchar et al., 1996) and in extrahepatic tissues such as uterus (Chakraborty et al., 1990; Paria et al., 1990; Liehr et al., 1995), breast (Telang et al., 1991; Liehr and Ricci, 1996), placenta (Liehr et al., 1995) and ovary (Hammond et al., 1986; Muskhelishvili et al., 2001). Thus, it is possible that PCBs may be metabolized by these enzymes and/or interfere with estrogen metabolism in these extrahepatic tissues by inducing CYP activities.

Furthermore, during PCB metabolism, the formation of reactive oxygen species (ROS) has been detected in cells in culture (Slim et al., 1999, 2000), and in laboratory animals (Pelissier et al., 1990; Saito, 1990). Oxidative stress or DNA damage may act as an initiator of the caspase cascade and apoptosis (Robertson and Orrenius, 2000). Apoptosis is a natural process by which follicles in the ovary degenerate (Hughes and Gorospe, 1991). Caspases are key effector components of apoptosis (Thornberry, 1998). The presence of caspase-3 was shown in granulosa cells of atretic follicles (Boone and Tsang, 1998; Berardinelli et al., 2004). Robles et al. (1999) showed that healthy granulosa cells possess the inactive form of caspase-3, whereas apoptotic granulosa cells possess the active enzyme.

We previously reported that PCB3 and its monohydroxylated and dihydroxylated metabolites 4-OH- and 3,4-diOH-PCB3 at concentrations of 0.06–60 ng/ml significantly increased estradiol levels in the culture medium of granulosa and theca cells derived from follicles of mature animals (Ptak et al., 2005). The rank order of potency in estradiol secretion was 3,4-diOH-PCB3 > 4-OH-PCB3 > PCB3. We also showed that this effect is in part due to increased aromatase activity (Ptak et al., 2006). The current studies were designed to test the hypothesis that porcine ovaries contain CYP1A1, CYP1A2 and CYP2B monooxygenases which could bioactivate PCBs. Moreover, we wished to compare the effects of PCB3 and its metabolites,