The Effects of Organochlorine and Metabolite Compounds on Aromatase (CYP19) Activity and Cytotoxicity *In Vitro* in Human Placental JEG-3 Choriocarcinoma Cells

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Introduction

Elevated concentrations of persistent environmental contaminants have been detected in biota, including humans, that are known or suspected as being disruptive to endocrine processes [1]. 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), other dioxin-like organochlorines (OCs) and non-dioxin-like OCs are known to elicit endocrine effects involving the estrogen- and androgen-hormone receptors, and also via the Ah-receptor-mediated and the subsequent regulation of cytochrome P450 (CYP) 1A enzymes. We suggest that CYP enzymes mediating the synthesis and metabolism of endogenous steroid hormones (CYP5 to 27 subfamilies) may be affected by persistent OCs.

CYP19 (aromatase) mediates the aromatization of androgens to estrogens, and is expressed in several human tissues including the ovaries, testes, adipose, and several sites in the brain [2]. However, the human placenta possesses the highest expression of CYP19 activity, and thus controls the production and balance of estrogenic steroids *in utero*. CYP19 also plays a functional role in sexual development and reproduction. The human placental JEG-3 choriocarcinoma cell line has been used to investigate biomarkers of placental toxicity *in vitro*. For example, CYP19 activity in JEG-3 cells was found to be inhibited by TCDD and dioxin-like PCB-126 (3,3',4,4',5-Cl_5) [3]. In this cell line, treatment with benzo[*a*]pyrene (B[*a*]P), and not TCDD has been associated with altered cell proliferation, and can elicit a decrease in epidermal growth factor receptor (EGFR) protein and the mRNA levels of the apoptosis-controlling *c-myc* protein, while increasing

the levels of transforming growth factor (TGF-1β) [4,5]. Reports on the endocrine disrupting capacity or mode of action are currently unknown for the biotically persistent methyl sulfonyl- ($MeSO_2$-) containing metabolites of PCBs, and tris(4-chlorophenyl)-methane (TCPMe) and -methanol (TCPMeOH). In the present study, we investigated the potential compound- and treatment-dependent effects *in vitro* of hydroxyl- and several $MeSO_2$-PCB metabolite compounds, their parent PCBs, TCPMe and TCPMeOH on JEG-3 cell proliferation and cytotoxicity, and on the CYP19 enzyme activity. The influence of JEG-3 cell confluency and the presence of serum in the incubation medium was also examined. The possibility of an OC-induced/-mediated apoptotic mechanism for the observed cytotoxicity was investigated.

Materials and Methods

The culturing of JEG-3 cells has been described in detail elsewhere [3,6]. Briefly, JEG-3 cells were cultured in 12-well plates to ca. 80% confluency, followed by treatment with 0.1 % DMSO solutions of DMEM cell culture medium (i.e., incubation medium), with and without the addition of 10% fetal calf serum (FCS). The final concentrations of the TCPMe, TCPMeOH, PCBs (52 (2,2′,5,5′-Cl_4) and 70 (2,3′,4′,5-Cl_4)), 4-hydroxy-2′,4′,6′-CB (4-OH-CB30) and $MeSO_2$-PCB (3- and 4-$MeSO_2$-containing pairs of PCB-52, -70, -87 and -101) treatments were generally 0.1, 1.0, 10, 100, 1000 and 10000 nM. The negative controls contained 0.1% DMSO only. All assays were performed (at least) in triplicate.

The CYP19 activity of the OC-treated and control JEG-3 cells was determined using the tritium release assay described by Lephart and Simpson [7] and Drenth *et al.* [3] with some modifications. The protein levels were determined from the same cell wells. Separate dose-response experiments were performed to determine the effects of OC treatments on the percent lactate dehydrogenase (LDH) leakage (a measure of cytotoxicity) from the JEG-3 cells. For some compounds, the treatment-dependent effect on the amount of DNA was measured. Immunochemical staining (fluorescence) was performed using a monoclonal antibody (APO2.7) which appears to be specific to a novel epitope on a mitochondrial membrane protein of Jurkat human T cells undergoing apoptosis [8].

Results and Discussion

With the exception of 4-$MeSO_2$-CB52 at the 10 μM treatment level, no significant ($P < 0.05$) protein-corrected decrease in CYP19 activity from controls was observed for the compounds and the treatment levels tested. However, for several of the OC compounds tested, a concomitant, treatment-dependent decreasing effect occurred for the protein-uncorrected CYP19 activity, and the protein and DNA levels. For example, these effects commenced for 4-OH-CB30 at ca. 1.0 μM (Figure 1a). Conversely, an increase in the % LDH leakage of JEG-3 cells occurred (Figure 1a). The same effects were observed for TCPMe and TCPMeOH at ca. 1.0 nM and ca. 1.0 μM, respectively. For 4-$MeSO_2$-

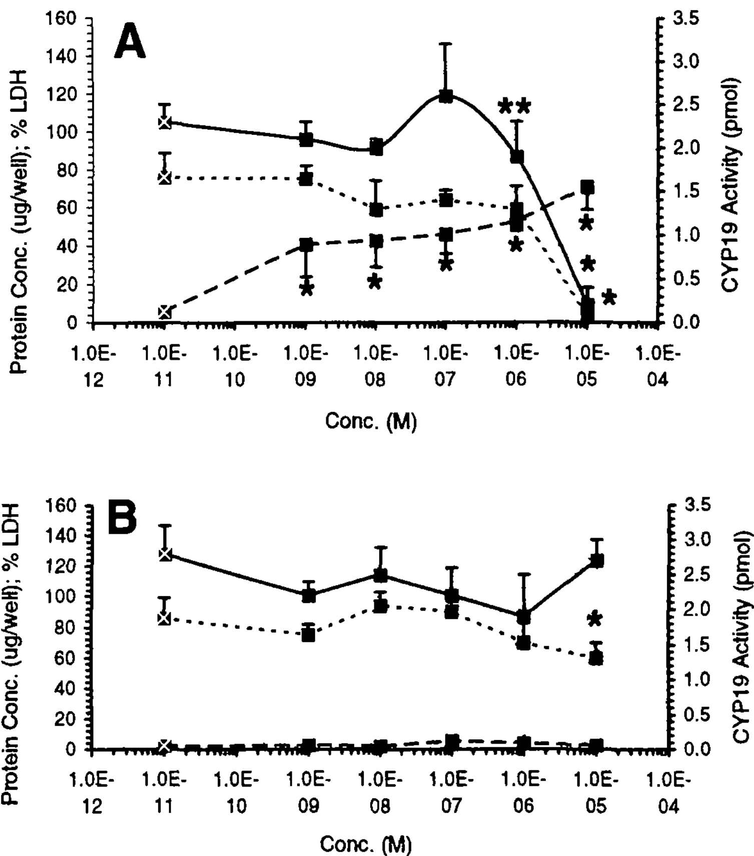

Figure 1. The effect of various concentrations of 4-OH-CB30 on CYP19 activity (solid line), protein level (small dashed line) and % LDH leakage (large dashed line) for human placental JEG-3 choriocarcinoma cells incubated in A) the absence of FCS and B) in the presence of 10 % FCS. An ANOVA for multiple comparisons, and using a Studentized Newman-Keuls posthoc test was used to determine whether the treated cells were significantly different from the controls (***P* < 0.01, ****P* < 0.05**). The control assays are designated by an 'x'.

CB52, -CB70 and -CB101 (2,2',4',5,5'-Cl_5), similar effects occurred at levels as low as 1.0 nM. For the 3-$MeSO_2$-containing pairs, no significant effect could be seen on CYP19 activity, protein level or % LDH leakage. For PCB-70, the protein drop and %LDH leakage commenced at ca. 1.0 nM, and the CYP19 activity began to decrease at 10 nM. For PCB-52, the effect on these parameters was much more marginal. For 3- and 4-

$MeSO_2$-CB87 (2,2',3',4',5-Cl_5), the CYP19 activity and protein decrease commenced at ca. 10 μM, whereas %LDH leakage commenced at ca. 1.0 nM.

In the case of 4-OH-CB30 (Figure 1b) and TCPMeOH, these effects could be largely negated by the presence of 10 % FCS in the incubation medium. It was clear that the cytotoxic effect on JEG-3 cells was compound-, treatment level-, and serum-dependent. Therefore, the JEG-3 cell line was found to be rather unstable towards the response of aromatase activity by xenobiotic treatment, as the response could be modulated by the presence of serum or the state of the confluency of the cells. Other compounds including B[*a*]P, TCDD and PCB-126 are currently being tested. In addition, an apoptotic mode of action for the OC-induced/-mediated cytotoxicity in the JEG-3 cells was suggested from the observed binding of the APO2.7 antibody [8)], after treatment with 10 and 50 nM of TCPMe and 4-$MeSO_2$-CB70, respectively. The qualitative intensity of fluorescence in the OC-treated cells was intermediate to the negative control cells and positive control cells (17β-estradiol- (2 μM) and dexamethasone- (10 μM) treated). 17β-estradiol and dexamethasone are known apoptosis-inducing agents *in vitro* in human Jurkat cells and rat fibroblasts [8,9)]. In summary, the use of the JEG-3 cells is not recommended for determining whether xenobiotics are inducers or inhibitors of CYP19 aromatase activity.

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