Effects of TCDD and PCB#126 on aromatase (CYP19) activity in the human choriocarcinoma cell line JEG-3.

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1. Introduction

The conversion of androgens to estrogens is catalyzed by an enzyme complex known as aromatase[1]. This complex consists of two components. The first is the microsomal cytochrome P450 enzyme named CYP19, the product of the *CYP*19 gene. The second component is a flavoprotein, NADPH reductase, which is responsible for transferring reducing equivalents to any cytochrome P450 enzyme present in the membrane of the endoplasmatic reticulum. The JEG-3 cell line has been extensively used for studying the placental function and exhibits several characteristics of normal trophoblasts, e.g. a high aromatase activity. Both JEG-3 cells and normal trophoblasts exhibit high aromatase activity. However, differences between normal trophoblasts and JEG-3 cells with respect to regulation of aromatase activity have also been observed[2),3)].
Several studies have revealed that TCDD and non-ortho PCBs exert anti-estrogenic effects in various species[4),5)].
This study was designed to examine possible effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3,3',4,4',5-pentachlorobiphenyl (PCB#126) on aromatase activity using the human choriocarcinoma cell line JEG-3. It was hypothesized that changes in aromatase activity by dioxin-like compounds could affect (local) concentrations of estrogenic or androgenic steroid hormones, and thus leading to (anti)-estrogenic effects.

2. Methods

Cell culture and conditions for treatment with TCDD and PCB#126.
JEG-3 choriocarcinoma cells were a kind gift of Prof. H.P.J. Bloemers, Department of Biochemistry, University of Nijmegen. These cells were maintained at 37° C in DMEM (Sigma) containing 10% heat inactivated fetal calf serum (FCS). The media were refreshed twice a week and cells were passaged by trypsinization every week.
JEG-3 cells from confluent T-75 flasks were trypsinized and resuspended in culture medium. The cells were seeded in twelve-well culture plates at a split ratio of 1:10 per unit of area. TCDD and PCB#126 solutions were prepared as 1000-fold stock solutions in dimethylsulfoxide (DMSO). Incubation medium was prepared just prior to incubation. After 2 days (approx. 40% confluent wells) media were replaced by incubation media. Every incubation was performed in triplo for the EROD measurement and in quadruplo for the aromatase activity assay. After 18 hrs of incubation, the cells were assayed for EROD or aromatase activity. Effects of TCDD and PCB#126 on EROD and aromatase activity were investigated in cells incubated with or without 10% FCS, since serum

can affect regulation of aromatase activity[1]. After the enzyme assays the protein content of the wells (cells lysed with 0.1 M NaOH) was measured using the method of Bradford with bovine serum albumin as standard.

Aromatase assay.
[1β-^{3}H]androstenedione was purchased from NEN-products, DuPont. The assay of aromatase activity was that described by Lephart and Simpson[1], in which aromatase activity was determined measuring the amount of ^3H_2O formed during aromatization of ^{3}H-androstenedione. Aromatase activity was inhibited for 91% when co-incubated with the aromatase inhibitor 4-hydroxyandrostenedione (Sigma) at concentrations higher than 20 nM.

EROD assay
The EROD activity assay was that described by Burke and Mayer[6], with some modifications. After 30 min of incubation with ethoxyresorufin, the amount of resorufin in the medium was determined using a fluorescence multiwell platereader (48 wells-plate).

3. Results

EROD activity
EROD activity increased strongly in the cells in a dose-dependent way for both toxicants with or without FCS. When corrected for the amount of protein per well, EROD activity was found to be increased by a factor 4.9 after treatment of the cells with TCDD in culture medium supplemented with 10% FCS. The maximum increase in EROD activity was 3.8-fold, when cells were treated with PCB#126 (fig. 1). In cells treated with TCDD or PCB#126 in serum-free culture medium, EROD activity increased by a factor of 4.7 and 5.1, respectively. Calculated EC_{50} values for TCDD and PCB#126 are given in table 1.

Aromatase activity
Aromatase activity, when corrected for the amount of protein, decreased in a dose-dependent way in presence and in absence of FCS for both TCDD and PCB#126 treated cells (fig. 2 and 3). Aromatase activity of cells treated with TCDD in presence of FCS decreased by a factor 1.9, whereas aromatase activity in absence of FCS decreased by a factor 4.9. For cells treated with PCB#126 these values were 1.7 and 2.6, respectively. Calculated EC_{50} values for TCDD and PCB#126 are given in table 1.

Protein content of the wells
As shown in fig. 4, the amount of protein per well, also decreased in a dose dependent way in the lower nM range after treatment with TCDD or PCB#126 in the presence or absence of FCS. The results shown are those of the cell cultures used previously for the aromatase activity. Effects on protein content of the cell cultures used after the EROD assay were comparable. The protein content decreased dose-dependently after treatment with TCDD, in the presence or absence of FCS by a factor 1.8 and 1.7, respectively. For PCB#126 treated cells these values were 1.9 and 2.5, respectively. Calculated EC_{50} values for TCDD and PCB#126 are given in table 1.

No dose-dependent effects on confluence of the wells, and thus on the number of cells per well, were observed by microscopic examination. All cells which were incubated for 18 hours in the absence of FCS appeared to have ceased growing in this period. The effects of both toxicants on cell proliferation are presently under further investigation in our laboratory.

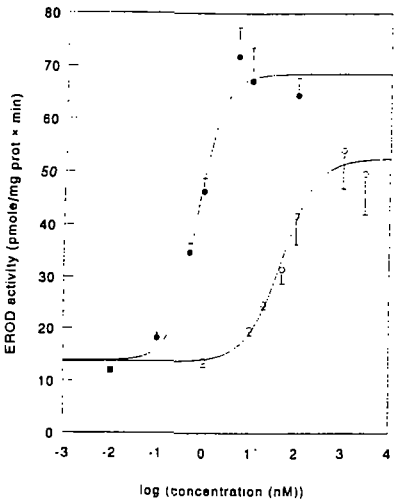

Fig. 1. EROD activity in JEG-3 cells (n=3) after incubation with TCDD (●) (control (■)) or PCB#126 (○) (control (□)). Sigmoidal curvefit shown.

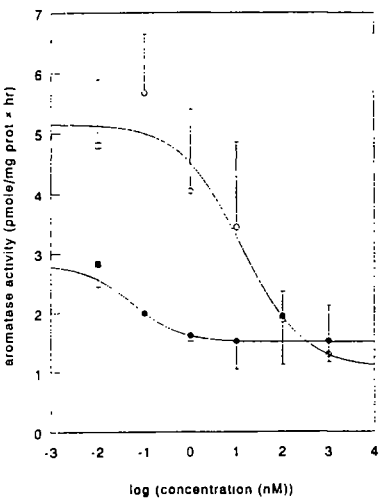

Fig. 2. Aromatase activity in JEG-3 cells after incubation with TCDD in presence (●) (control (■)) or absence (○) (control (□)) of Fetal Calf Serum (n=4). Sigmoidal curvefit shown.

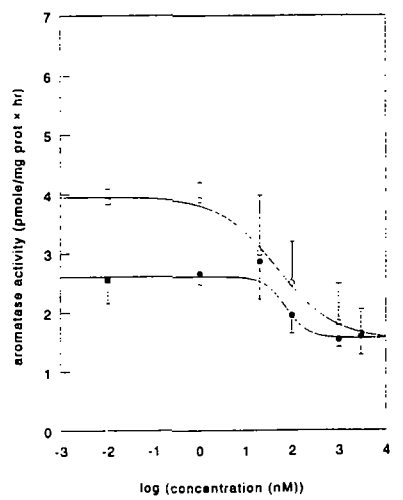

Fig. 3. Aromatase activity in JEG-3 cells after incubation with PCB#126 in presence (●) (control (■)) or absence (○) (control (□)) of Fetal Calf Serum (n=4). Sigmoidal curvefit shown.

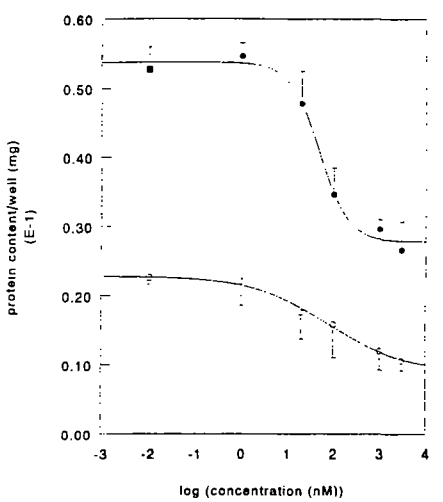

Fig. 4. Protein content of wells with JEG-3 cells after incubation with PCB#126 in presence (●) (control (■)) or absence (○) (control (□)) of Fetal Calf Serum (n=4). Protein content determined after aromatase activity assay. Sigmoidal curvefit shown.

Table 1. EC_{50} values (nM) and 95% confidence intervals (nM) for effects of TCDD and PCB#126 on EROD and aromatase activity and protein content in JEG-3 cells, using a sigmoidal curvefit.

	EROD activity		aromatase activity		protein content	
with FCS	EC_{50}	95% conf. int.	EC_{50}	95% conf. int.	EC_{50}	95% conf. int.
TCDD	0.71	0.53-0.94	0.052	0.0053-0.51	0.033	0.0042-0.25
PCB#126	48	29-80	75	31-183	48	28-84
without FCS						
TCDD	0.40	0.094-1.7	13	0.82-208	2.6	0.21-33
PCB#126	20	9.0-46	48	5.4-425	77	$1.2\text{-}4.9\cdot10^{3}$

4. Discussion and Conclusions

Following 18 hrs exposure to TCDD or PCB#126, the protein content of JEG-3 cells grown in 12 wells plates decreased already at 10-100 nM concentration levels. This effect is clearly dose dependent and observed in the presence as well as in the absence of FCS during incubation. Because confluence of wells appeared not to be affected by both toxicants and JEG-3 cells appeared to have stopped growing in the absence of FCS, it is likely that the decrease in protein content per well is a result of a decrease in protein content per cell, not a decrease in the number of cells.
Even when corrected for protein content, there is still a pronounced dose-dependent decrease in aromatase activity by TCDD and PCB#126 treatment. Whether or not this is a concomitant effect of the dose-dependent decrease in protein content of the cells, needs to be further investigated. In spite of the observed decrease in protein content the EROD activity remained maximally induced, even after correction for protein content of the wells.
The observed induction of EROD activity is in agreement with studies using human placenta. Okey et al.[7] have reported detectable levels of *Ah* receptor in human placenta. In a study of Pasanen[8], smoking mothers appeared to have higher EROD activity in placental microsomes than non-smoking mothers. The fact that EROD activity in JEG-3 cells is induced after incubation with TCDD or PCB#126, indicates that these cells are highly responsive towards dioxin-like compounds.

The maximum effect of both toxicants on aromatase activity was found in the groups without FCS, while maximum effects on protein content appeared not to be influenced by the presence of FCS. Relative Potency Values, defined as the ratio of EC_{50} values of TCDD and PCB#126, in the presence or absence of FCS were $1.5\cdot10^{-2}$ and $2.0\cdot10^{-2}$, $6.9\cdot10^{-4}$ and $2.7\cdot10^{-1}$, and $6.9\cdot10^{-4}$ and $3.4\cdot10^{-2}$, for EROD activity, aromatase activity or the protein content, respectively. These values are lower than the commonly used TEF value for PCB#126 of 0.1[9),10)]. However, these Relative Potency Values have limited value, because of differences in slope and maximal effect of the TCDD and PCB#126 treated cells.

Whether (anti)-estrogenic effects of TCDD and related compounds could be a result of effects on (local) estrogen concentrations, by modulating CYP19 expression, needs further investigation. The results of our study demonstrate that such effects cannot be excluded anymore.

5. References

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