EFFECTS OF DIOXIN-LIKE COMPOUNDS ON ESTROGEN METABOLISM IN MCF-7 AND MCF-10A CELL LINES

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Introduction

Dioxin-like compounds are a group of widespread environmental pollutants and include polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins- and furans (PCDDs/PCDFs), that exert a broad spectrum of biological and toxic effects similar to that of the most potent congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[1, 2]. These compounds are often studied for their possible role in the etiology of cancer. However, epidemiological studies are ambiguous. Studies with women exposed to TCDD in a plant accident in Seveso, Italy appear to have a decreased breast cancer risk in the fist decade following the accindent[3]. Recent studies, however, show a correlation between increased TCDD serum levels and an increased breast cancer risk for women exposed to TCDD[4].

We hypothesized that a possible correlation between dioxin-like compounds and increased breast cancer risk might be a result of their ability to affect cytochrome P450 enzymes. Dioxin-like compound actions are mediated through the aryl hydroxarbon receptor (AhR) which also regulates cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) expression. In human mammary tissue, CYP1A1 and CYP1B1 are the main enzymes responsible for estrogen hydroxylation to 2- and 4-hydroxyestrogens, respectively. Unless inactivated, the hydroxyestrogens may undergo redox cycling and form reactive quinones that can bind to macromolecules such as DNA. Quinones from the 2-hydroxyestrogens can form stable DNA adducts, but quinones from the 4-hydroxyestrogens might form depurinating DNA adducts, a potential tumor-initiating event in carcinogenesis[5]. In *ex vivo* studies with human mammary and uterine tissues, the 4-/2-hydroxyestrogen ratio appeared to be a marker for the prensence of a neoplasm; a higher ratio of 4-/2-hydroxyestrogens was observed in malignant tumor tissues compared with healthy tissues[6, 7]. In this study, we investigated the effects of several dioxin-like compounds on estrogen metabolism in a malignant (MCF-7) and a non-tumorigenic (MCF-10A) human mammary cell line. Special emphasis was placed on the effects of these compounds on the estrogen 4-/2-hydroxylation ratio and the use of this ratio as predictive marker for the development of mammary neoplasms.

Methods and Materials

MCF-7 and MCF-10A cell cultures were exposed to various concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126) or 3,3'4,4',5,5'-hexachlorobiphenyl (PCB 169), for 72 hours. Then, medium was replaced with serum-free medium containing 1 µM estradiol (E_2). After 6 hours, a 1-ml aliquot of the medium was taken and the internal standard (200 pmol) was added.

Estrogen metabolites were extracted with dichloromethane and trimethylsilyl derivatives were prepared. After evaporation, the residues were dissolved in 20 µl cylohexane and 1 µl was injected and analyzed by GC/MS (DB-5MS 30 m x 0.25 mm column with a film thickness of 0.25 µm) (method adapted after Spink et. al[8]) . Concentrations of estradiol, estrone (E_1), methoxyestradiols (4- and 2-$MeOE_2$) and methoxyestrones (4- and 2-$MeOE_1$) were calculated using the peak area at *m/z* 416, 342, 446 and 372, respectively, and corrected with the peak area of the internal standard (equilin) at *m/z* 340. Peak identification was performed by using the corresponding standards.

mRNA isolation and quantification of CYP1A1 and CYP1B1 mRNA expression was determined after 72 hours of incubation of the cells with TCDD (10 nM), PCDF (10 nM), PCB 126 (1 µM), PCB 169 (5 µM). RT-PCR conditions and primers are described by Sanderson et al. [9].

Results and Discussion

2- and 4-methoxyestrogen ($MeOE_{1/2}$) formations were used as measures of the estrogen 2- and 4-hydroxylation pathways, respectively, because of their greater stability and lower detection limits then the hydroxyestrogens.

TCDD, PCDF, PCB 126 concentration-dependently induced 2-$MeOE_{1/2}$ formation in MCF-7 and MCF-10A cells (table 1 and figure 1). PCB 169 induced 2-$MeOE_{1/2}$ formation in both cell lines at the highest concentration tested (5 µM), but no EC_{50} value could be obtained. Effects on 4-$MeOE_{1/2}$ formation were less pronounced in both cell lines. Generally, 4-$MeOE_{1/2}$ formation increased at the lower end of the concentration curve, but the induction decreased somewhat upon strong increase of 2-$MeOE_{1/2}$ formation. Only TCDD caused a concentration-dependent induction with EC_{50} values of 0.02 and 0.3 nM in MCF-7 and MCF-10A, respectively. PCB 169 was an inhibitor of 4-$MeOE_{1/2}$ formation with IC_{50} values of 0.7 and 2.2 nM in MCF-7 and MCF-10A, respectively. The inhibitory effects of PCB 169 were selective for CYP1B1 activity as was shown by its ability to inhibit TCDD-induced 4-$MeOE_{1/2}$ formation at concentrations were no effects on 2-$MeOE_{1/2}$ formation were observed.

The constitutive 4-/2-$MeOE_{1/2}$ ratios were 2.99 ± 0.78 and 0.93 ± 0.40 in MCF-7 and MCF-10A, respectively. These ratios were comparable with estrogen 4-/2-hydroxylation ratios found in malignant and healthy *ex vivo* breast tissue[6, 7]. Incubation with dioxin-like compounds resulted in a concentration-dependent decrease in the 4-/2-$MeOE_{1/2}$ ratios (EC_{50} values in table 1).

The induction of estrogen metabolism could be attributed to induced CYP1A1 and CYP1B1 expression, as was confirmed by induced mRNA expression of both enzymes after incubation with the dioxin-like compounds. The inhibitory effects of PCB 169 on estrogen metabolism showed to be a direct catalytic inhibition of CYP1B1 activity, since no effects on CYP1B1 mRNA expression could be observed.

Conclusions

Incubation with dioxin-like compounds resulted in a concentration-dependent decrease in the 4-/2-$MeOE_{1/2}$ ratio, but an increase in potentially carcinogenic estrogen metabolites in both MCF-7 and MCF-10A cells. This indicates that even though the estrogen 4-/2-hydroxylation ratio may be used as indicator for the presence of neoplasms, it is readily lowered by dioxin-like compounds and its value as a prognostic parameter for cancer risk should be further examined.

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Table 1: EC_{50} values (nM) and relative toxicological potencies for induction of 2-MeOE2 formation and decrease in 4-/2-MeOE2 ratio in MCF-7 and MCF-10A cells.

		MCF-7		MCF-10A	
Compound	TEF values	2-MeOE2 formation	Decrease ratio	2-MeOE2 formation	Decrease ratio
TCDD	1	0.05 (1)	0.022 (1)	1.2 (1)	0.79 (1)
PCDF	0.5	0.1 (0.5)	2.18 (0.01)	0.4 (3)	21.5 (0.04)
PCB 126	0.1	7.6 (0.01)	0.075 (0.3)	446 (0.05)	0.18 (4.4)
PCB 169	0.01	NA	0.079 (0.3)	NA	0.69 (1.1)

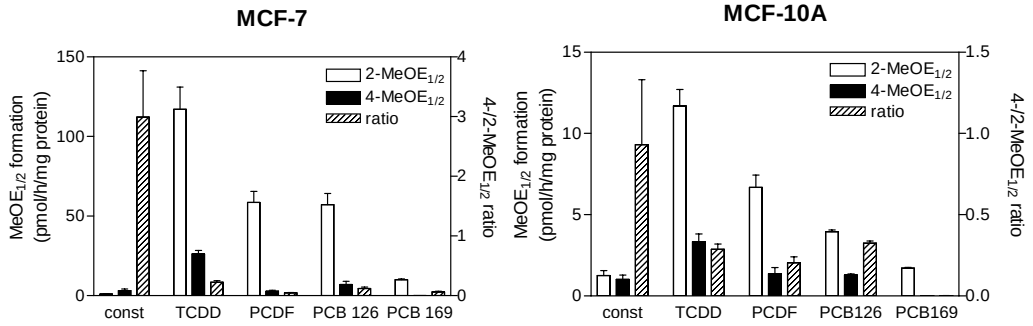

Figure 1. 2- and 4-$MeOE_{1/2}$ formation (pmol/h/mg protein) and 4-/2-$MeOE_{1/2}$ ratio in MCF-7 (left panel) and MCF-10A cells (right panel) after incubation with TCDD (10 nM), PCDF (10 nM), PCB 126 (1 µM), PCB 169 (5 µM) or the solvent vehicle (DMSO, 0.1% v/v)