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DIFFERENCES IN THE ACTION OF TCDD AND PCNS ON AHR AND CYP1A1 EXPRESSION IN MCF-7 BREAST CANCER CELL LINE

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INTRODUCTION

Polychlorinated naphthalenes (PCNs) are group of diaromatic compounds composed of two fused benzenes that contain one to eight chlorines per molecule and form 75 possible congeners[1,2]. PCNs were sold as a variety of mixtures (technical formulations), which composed of a lower molecular weight from mono- to trichloronaphthalenes (Halowax 1000 and 1001) to predominately of octachloronaphthalene (Halowax 1051).

Toxic episodes of accidental animal and occupational human poisonings with PCNs took place after exposure to mixtures containing predominately penta- and hexachloronaphthalenes (Halowax 1014)[3].

The higher chlorinated technical PCN mixtures produce biological effects largely similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and including induction of the liver cytochrome P450-associated enzymes, ethoxyresorufin-*O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH).[4]

Hootha et al. (2012) assessed the toxicity and potency of the chlorinated naphthalenes 1,2,3,4,6,7-hexachloronaphthalene (PCN 66) and 1,2,3,5,6,7-hexachloronaphthalene (PCN 67) relative to that of TCDD. Despite the fact that PCN 66 and PCN 67 exposure resulted in biochemical and histopathologic changes similar to those seen with TCDD - which could suggest that these compounds be included in the WHO Toxic Equivalency Factor (TEF) scheme - the estimated relative potency values obtained predicted that these hexachlorinated naphthalenes would not contribute greatly to the overall human body burden of dioxin-like activity.

AIM

To better evaluation of a wide range of PCN congeners for possible inclusion in the TEF scheme (Van den Berg et al., 2006), we exposed human MCF-7 breast cancer cells for 72 h to PCN 34, 39, 42, 46, 48, 52, 53, 54, 66, 67, 70, 71, 73 or 74, and compared their induction of toxicological and biochemical endpoints to that of TCDD. As endpoints, we evaluated AhR protein expression, CYP1A1 activity and protein expression.

MATERIAL AND METHODS

Cell culture

MCF-7 human breast cancer cells (ATCC, Manassas, VA, USA) were routinely cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 IU/ml of penicillin and 100 µg of streptomycin. Cells were seeded into 96-well culture plates at a density of 2.5 x 10^4 cells/well and allowed to attach overnight. The next day, the medium was changed and cells were treated with 1000 pg/ml PCN (34, 39, 42, 46, 48, 52, 53, 54, 66, 67, 70, 71, 73, and 74) or 1 nM TCDD (positive control) for an additional 6 and 12 h (to determine CYP1A1 activity); 3, 6 and 12 h (to determine AhR protein expression) or for 12 and 24 h (to determine CYP1A1 protein expression).

CYP1A1 activity

The ethoxyresorufin-O-deethylase (EROD) assay, a specific measure of CYP1A activity, was performed as described by Kennedy and Jones (1994). The fluorescence of resorufin generated by the CYP1A-mediated conversion of ethoxyresorufin was measured at 15-min intervals for up to 2 h with a fluorescence plate reader (FLx 800; Bio-Tek) using a 530 nm excitation filter and a 590 nm emission filter. After 2 h, the protein concentration in each well was determined using a fluorescamine protein assay (Sigma Chemical Co.). Results were calibrated against a resorufin standard curve (0–100 nM) and a bovine serum albumin (BSA) standard curve (0–1000 µg). Science, Mannheim, Germany).

AhR and CYP1A1 protein expression

Blots were incubated overnight with primary antibodies specific to AhR (sc-8088; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and CYP1A1 (sc-9828; Santa Cruz Biotechnology, Inc.) diluted 1:200. After incubation with the primary antibody, the membranes were washed three times and incubated for 1 h with a horseradish peroxidase (HRP)-conjugated secondary antibody (sc-2020; Santa Cruz Biotechnology, Inc.) diluted 1:2000. Immunopositive bands were visualized using Western Blotting Luminol Reagent (Santa Cruz Biotechnology Inc.) and the ChemiDoc XRS+ System (Bio-Rad Laboratories Inc.). The relative levels of protein expression were determined using ImageJ analysis software (US National Institutes of Health, Bethesda, MD, USA). Individual protein levels were normalized to that of β-actin controls, and the ratio of protein to β-actin was normalized to that in the untreated control group (defined as 1).

DISCUSSION

To explore the mechanism of action of PCNs, we investigated the effects of individual PCN congeners on AhR receptor expression and induction of CYP1A1 activity and protein expression using a concentration selected on the basis of results of previously experiments concerning action on cell viability and DNA fragmentation.

Surprisingly, an analysis of AhR protein expression showed that TCDD had no effect on AhR protein expression at any concentration or exposure time tested but stimulated CYP1A1 activity at all concentrations tested but none of these concentrations affected CYP1A1 protein expression.

The aryl hydrocarbon receptor (AhR) is commonly described as a transcription factor, which regulates xenobiotic-metabolizing enzymes. One of the main problems is that there are many toxic phenomena that cannot be solely explained by the classical genomic model of the action of TCDD which is based on its ability to activate aryl hydrocarbon receptor (AhR) and induce detoxification enzymes and proteins, particularly cytochrome P450s (CYPs). Bin Dong (2009) produced the evidence the existence of a nongenomic pathway of TCDD-activated AhR, using MCF10A, an immortalized human mammary epithelial cell line, and MMDD1, a mouse kidney macula densa cell line, as cell models. This newly identified pathway was named as nongenomic pathway because the initial signaling takes place in the cytosol, and aryl hydrocarbon receptor nuclear translocator (ARNT), the AhR's dimerization partner necessary for the classical genomic model is not required in this nongenomic pathway.

Among the investigated PCN congeners, tetra- and heptachlorinated naphthalenes significantly stimulated CYP1A1 activity in association with induction of AhR protein expression after 12 h of exposure. With a longer duration of exposure, penta-, hexa-, and heptachlorinated naphthalenes also stimulated CYP1A activity. Most PCN congeners also induced an increase in CYP1A1 protein expression; however, this effect was maintained for 24 h only in the case of highly chlorinated naphthalenes. On the basis of concentration-response modeling of CYP1A1, CYP1A2 and thymic atrophy, Hootha et al. (2012) estimated the toxicity and potency of the chlorinated naphthalenes 1,2,3,4,6,7-hexachloronaphthalene (PCN 66) and 1,2,3,5,6,7-hexachloronaphthalene (PCN 67) relative to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and suggested that these PCN congeners should be included in the WHO TEF scheme, although the estimated relative potencies indicate that these hexachlorinated naphthalenes should not contribute greatly to the overall human body burden of dioxin-like activity.

Effects of PCNs on AhR protein expression

After 3 h of incubation, no congeners had a significant effect on AhR protein expression. At 6 h, the highly chlorinated naphthalenes PCN 53, 54, 66, 71, 73, and 74 significantly up-regulated AhR expression. After 12 h, only the low-chlorinated naphthalenes PCN 35, 39, 42 and 46 up-regulated AhR protein expression .

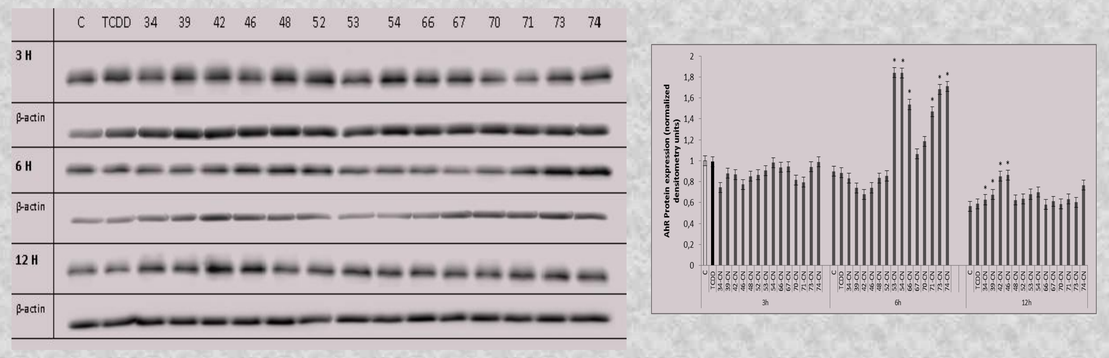

Fig. 1 Effects of TCDD (1 nM) and individual PCN congeners (1000 pg/ml) on AhR protein expression after 3, 6 and 12 h of incubation. Representative blots from three experiments are shown in the panels. All means marked with * ($p < 0.05$) are significantly different from control values.

Effects of PCNs on CYP1A1 activity

TCDD increased CYP1A1 activity t at 6 h and 12 h.
PCN 42, 46, 48, 52, 73, and 74 significantly increased CYP1A1 activity at 6h exposure . This increase was maintained at 24 hours for the PCN 46, 52, 73 and 74.

After 6h of exposure, the potency in CYP1A1 activity , of PCN 34,39, 53,54,66,67 and 70 was fivefold lower, and for PCN 46, 48, 52,71,73 and 74 twofold lower in comparison with TCDD.

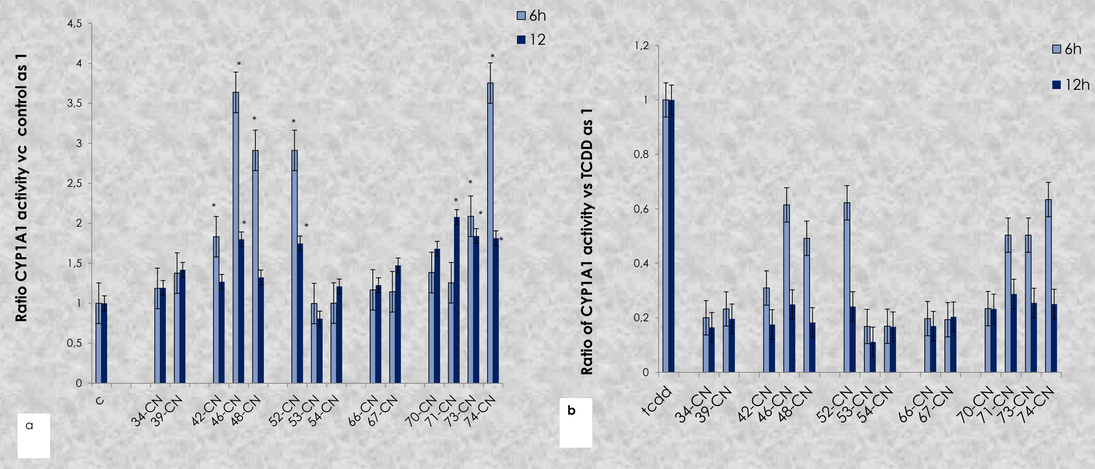

Fig.2. Time-dependent effects of individual PCN congeners on CYP1A1 activity determined by the EROD assay showed as a) ratio vs. control as 1 and b) ratio vs. TCDD as 1. All means marked with * ($p < 0.05$) are significantly different from control values.

Effects of PCNs on CYP1A1 protein expression

TCDD had no effect on CYP1A1 protein expression, while four low-chlorinated naphthalenes (PCN 34, 39, 42, and 46) and three highly chlorinated naphthalenes (PCN 70, 71, and 74) significantly increased CYP1A1 protein expression after 12-h exposure. This increase was maintained at 24 hours for the highly chlorinated naphthalenes PCN 52, 53, 54, 66, 67, 70, 71, and 74.

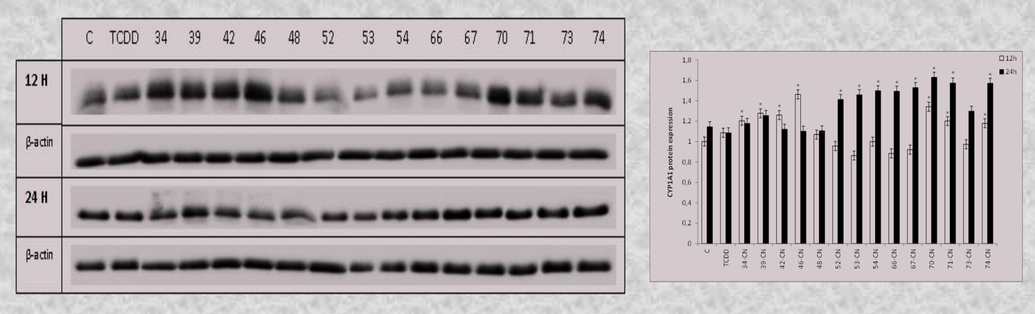

Fig. 3. Time-dependent effects of TCDD and individual PCN congeners on CYP1A1 protein expression. Representative blots from three experiments are shown in the panels. All means marked with * ($p < 0.05$) are significantly different from control values.

CONCLUSION

- This finding suggests different mechanism of action of PCNs and TCDD in breast cancer cells.
- We identified congeners that specifically induced the expression of AhR and CYP1A1 protein, which could generate active metabolites and further affect various cell processes.