COMPARISON OF THE AhR- DEPENDENT GENE EXPRESSION INDUCED BY SELECTED DIOXIN-LIKE COMPOUNDS IN RAT LIVER AND LUNG EPITHELIAL CELLS

Krckova S[1], Hulinkova P[1], Andersson P[2], Vondracek J[1], Machala M[1]

[1] Department of Toxicology, Pharmacology and Imunotherapy, Veterinary Research Institute, Brno, Czech Republic; [2] Department of Chemistry, Univeristy of Umeå, Umeå, Sweden

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are common persistent organic environmental pollutants, with 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) being the most toxic of polyhalogenated aromatic hydrocabons (1). Their broad spectrum of biological responses and adverse effects is primarily mediated via aryl hydrocarbon receptor (AhR) transactivation. This fact allowed to establish toxic equivalency factor (TEF) approach toxicity characterization of individual dioxin-like compounds (2). The toxicity of these compounds is expressed relative to a reference compound, TCDD. Several previous studies reported cell- and tissue-specific potencies of individual dioxin-like toxicants. We have previously observed partially different AhR-dependent responses e.g. between hepatoma and liver epithelial progenitor-like cells (3). The principal aim of our study was to compare the TEF values with relative potencies to induce AhR target genes (CYP1A1 and CYP1B1) after exposure to selected dioxin-like compounds in rat liver progenitor-like and rat lung epithelial cells, representing two significant tissue targets of dioxin-like compounds.

Material and methods

Cells. Rat liver epithelial WB-F344 cells (provided by James E. Trosko, MSU, East Lansing, MI) were cultivated in Dulbecco's Modified Eagle's Medium (Invitrogene, Carlsbad, CA) supplemented with 25 mM sodium bicarbonate, 10 mM HEPES and 5% heat-inactivated fetal bovine serum (PAA, Pasching, Austria). The cells were subcultured twice a week and for this study, they were used only at passages 12-20. Rat lung epithelial RLE-6TN cells were cultivated in Ham F12 medium(Gibco, UK) with further supplements (bovine putuitary extract, insulin, transferin, epidermal growth factor, insulin-like growth factor), antibiotics (gentamicin) and 5% heat-inactivated fetal bovine serum (PAA, Pasching, Austria).

Chemicals. TCDD, PCDDs, PCDFs and PCBs were prepared and cleaned with fractionation on a column packed with active carbon mixed with Celite.

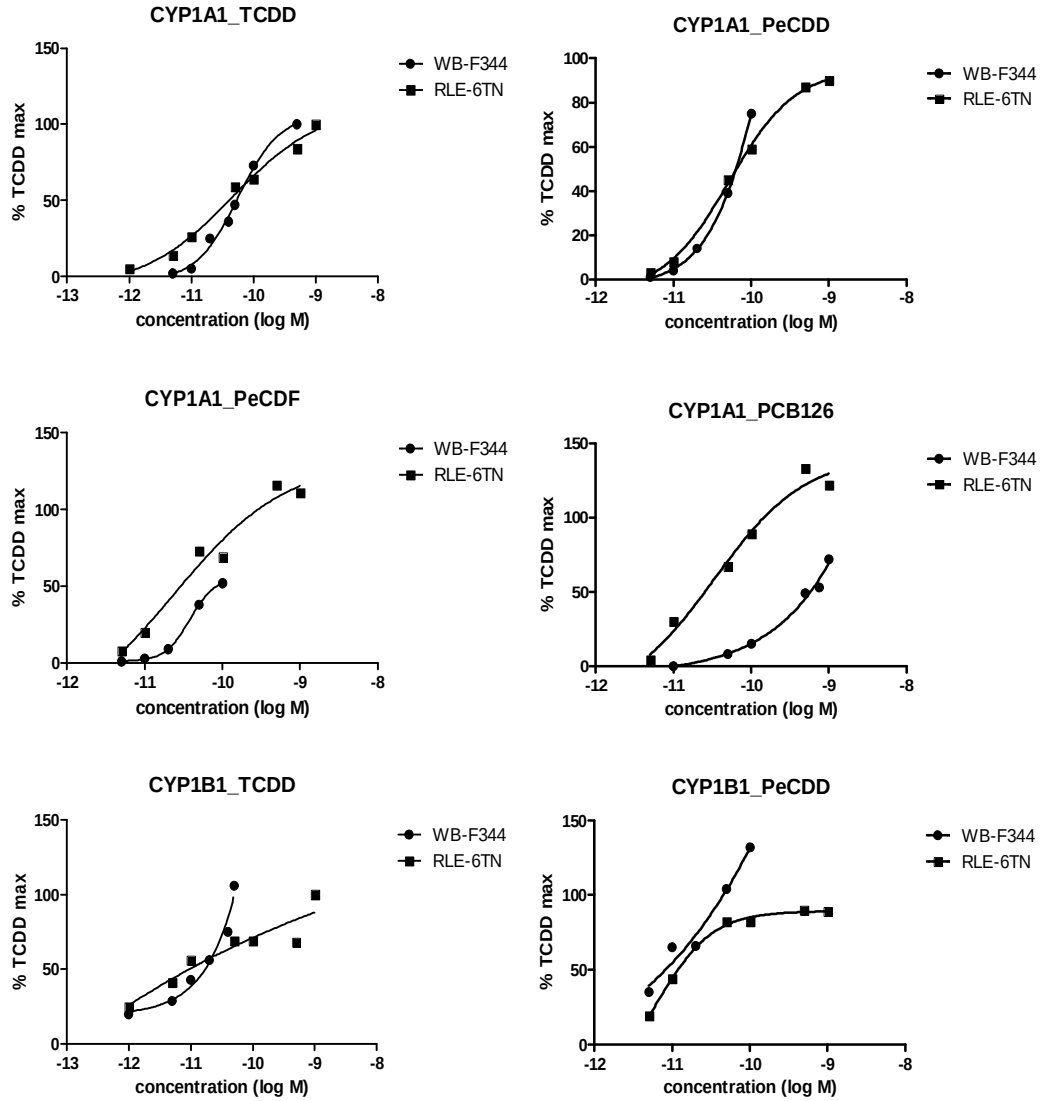
Real-time RT-PCR. Total RNA was isolated from cells using the NucleoSpin RNA II kit (Macherey-Nagel) according to the manufacturer's instructions. The amplifications of the samples were carried out using QuantiTect Probe RT-PCR kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's specifications. The sequence of probes and primers used as published previously (4). Gene expression for each sample was normalized to housekeeping gene porphobilinogen deaminase (PBGD).

Results and discussion

In this study we determined the dose-response curves of selected dioxin-like compounds. The expression of AhR-dependent genes *Cyp1a1* and *Cyp1b1* were determined by quantitative real time reverse transcription (qRT-PCR) assay detecting the respective mRNAs. The EC_{20} values were used for calculation of relative potencies (REP) in both epithelial cell lines. In the case of the *Cyp1a1* gene, the relative potencies derived in WB-F344 cells were comparable with the TEFs established by the WHO, with exception of PCBs 118, 156 and 167, which had all only a weak or no effect. Maximum induction were observed for the following congeners: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HexCDD, 1,2,3,4,6,7,8-HepCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 1,2,3,4,7,8-HexCDF, 2,3,4,6,7,8-HexCDF, 1,2,3,4,6,7,8-HepCDF, PCB 126, PCB 77 and PCB 169. The second group consisted of partial AhR agonists 1,2,3,4,7,8,9-HepCDF and a series of PCBs (congeners No. 74, 105, 156 and 189). Nevertheless, there were still deviations form the WHO TEF values; notably, the REP value for PCB 77 was two orders higher than established TEF value.

In a second step, the REP values were also developed for induction of CYP1B1 mRNA. Slightly lower REP values were found for PeCDD, 2,3,4,6,7,8-HexCDF and PCB 169, when compared to WHO TEF values, and PCB118 had only minimal effect on AhR-mediated Cyp1b1 expression.
Finally, the AhR-inducing potencies of selected congeners were determined in lung epithelial RLE-6TN cells. Data on induction of CYP1A1 and CYP1B1 mRNA in lung RLE-6TN cells are presented in Figure 1 (as compared to WB-F344 and the respective EC and REP values are summarized in Table 1. The REP values obtained in both liver progenitor-like cells and lung cells were comparable.

Figure 1. Induction of AhR-dependent genes CYP1A1 and CYP1B1 mRNA by selected dioxin-like compounds in rat liver epithelial WB-F344 and lung epithelial RLE-6TN cells



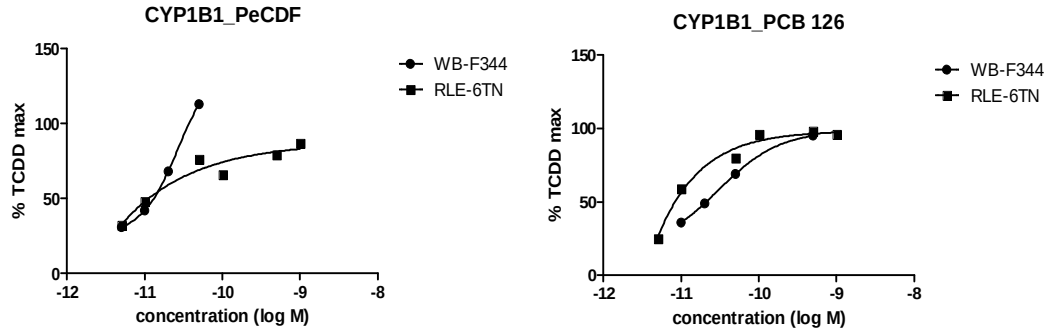


Table 1: Comparison of established TEFs with relative potencies of dioxin-like compounds (based on the expression of CYP1A1 mRNA and $EC_{20\ TCDD}$ values) in rat liver and lung epithelial cells

	WB-F344 EC_{20TCDD} (M)	**RLE-6TN** EC_{20TCDD} (M)	**WB-F344** REP (EC_{20})	**RLE-6TN** REP (EC_{20})	WHO 2005 **TEF**
TCDD	1.92E-11	4.704E-12	1.0	1.0	1.0
PeCDD	2.79E-11	1.575E-11	0.7	0.3	1.0
PeCDF	2.57E-11	7.864E-12	0.7	0.6	0.3
PCB 126	1.28E-10	1.916E-11	0.2	0.2	0.1

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